

# Modulation of uPA, MMPs and their inhibitors by a novel nutrient mixture in human colorectal, pancreatic and hepatic carcinoma cell lines



Publication from the Dr. Rath Research Institute

International Journal of Oncology | 2015 Vol. 47: 370-376



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## **M. Rath, M.D.**

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



# What Are Gastrointestinal (GI) Cancers

- ❑ Group of cancers that affect the digestive system
  
- ❑ Includes the following cancers:
  - Esophagus
  - Gallbladder
  - Liver
  - Pancreas
  - Stomach
  - Small intestine
  - Bowel (large intestine or colon and rectum)
  - Anus





# Cancers studied in our research paper



**1. Colorectal cancer**



**2. Pancreatic cancer**



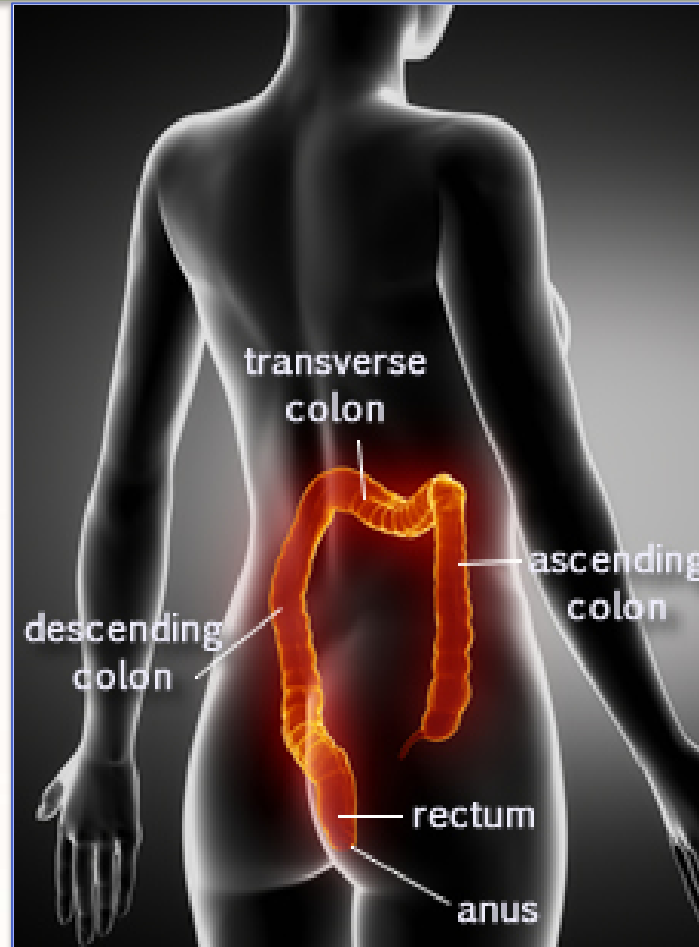
**3. Liver cancer**

# 1

# Colorectum is a part of our GI tract

## COLON

- Absorbs water, minerals, nutrients
- Nutrients mix with mucus and bacteria in the intestine, starting the formation of feces
- Bacteria break down the fiber to produce nutrients that nourish the cells that line the colon
- The residue (feces) is transferred to the rectum and anus by peristaltic movements

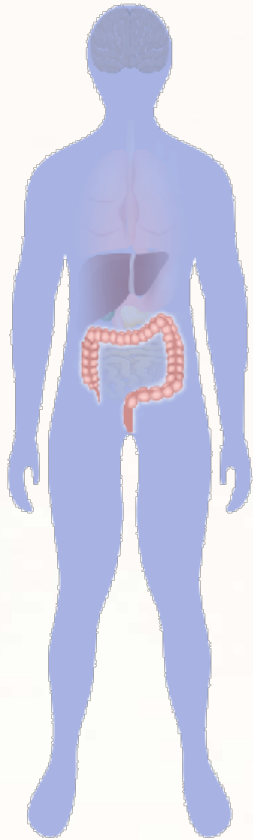


## RECTUM

- Final part of the digestive tract, opens into the anal canal
- Temporary storage of feces/ waste material
- Accumulated feces put pressure on the rectum walls, initiating defecation reflex and their movement to the anal canal

# 1

# COLORECTAL CANCER



The third most commonly diagnosed cancer and the third leading cause of cancer death (US, 2014).

Very treatable when detected early but once the cancer metastasizes to lymph nodes, liver or other areas, the 5-year survival rate is < 10%.

# 1

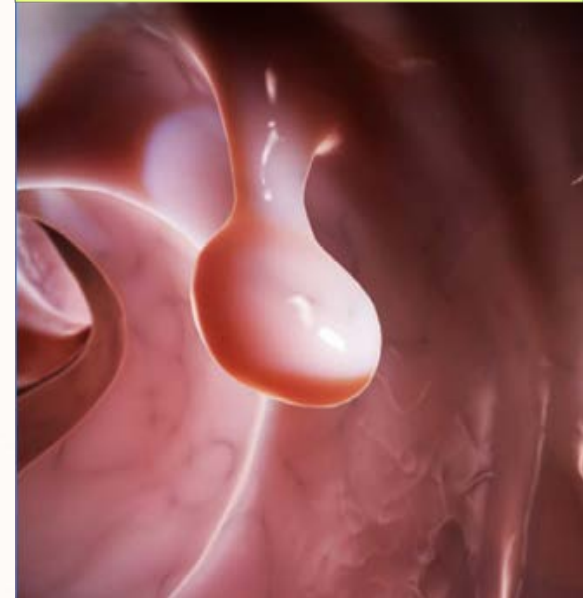
# COLORECTAL CANCER

## Symptoms:

- Change in bowel habits including diarrhea or constipation
- Rectal bleeding or blood in the stool
- Persistent abdominal discomfort, cramps, gas or pain
- Weakness or fatigue
- Unexplained weight loss

Most colorectal cancers start as a **polyp**, a small noncancerous clump of cells that develops at the inner wall of the colon or rectum. Over time, some of these polyps become colon cancers.

*Polyp: abnormal growth of tissue*



An abdominal organ, located behind the stomach

## Functions

### ❑ Exocrine:

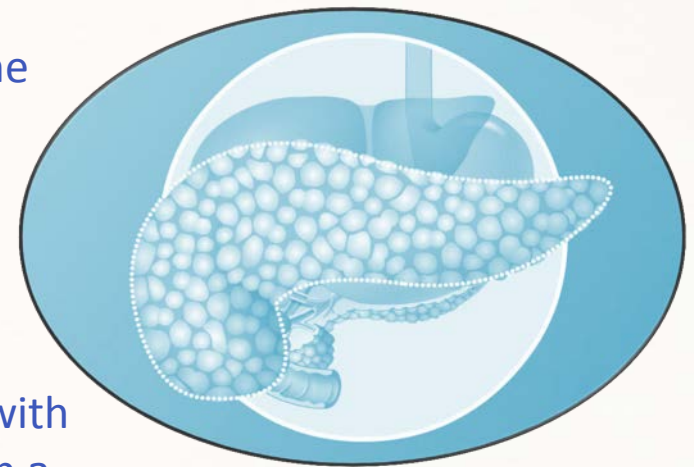
Produces digestive enzymes that break down proteins, fats and carbohydrates. It assists in the absorption of nutrients by the intestines.

### ❑ Endocrine:

Produces hormones such as insulin. Maintains healthy blood glucose and salt levels.

**Consequences of pancreas dysfunction:** problems with digesting food or maintaining the blood sugar within a healthy range.

- ❑ Diarrhea, bloating, flatulence
- ❑ Weight loss, malnutrition
- ❑ Poor blood sugar control, diabetes

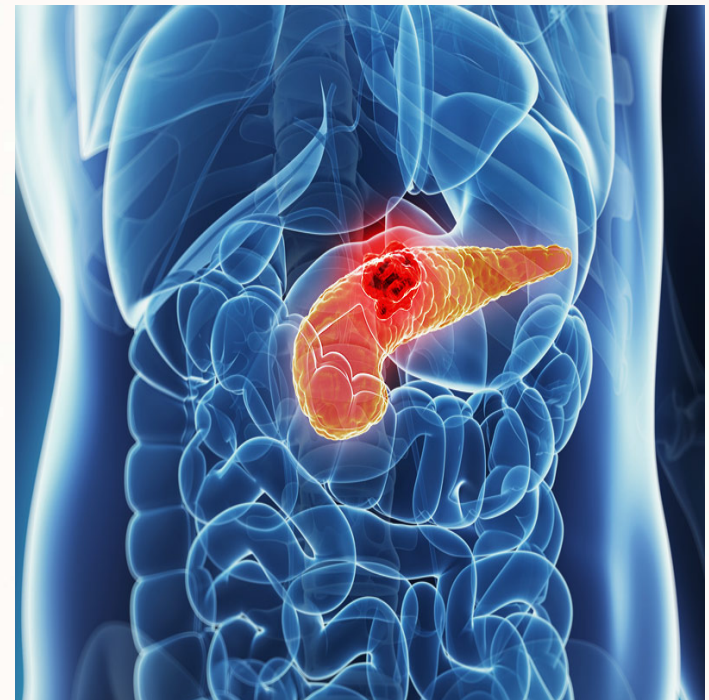




# 2

# PANCREATIC CANCER

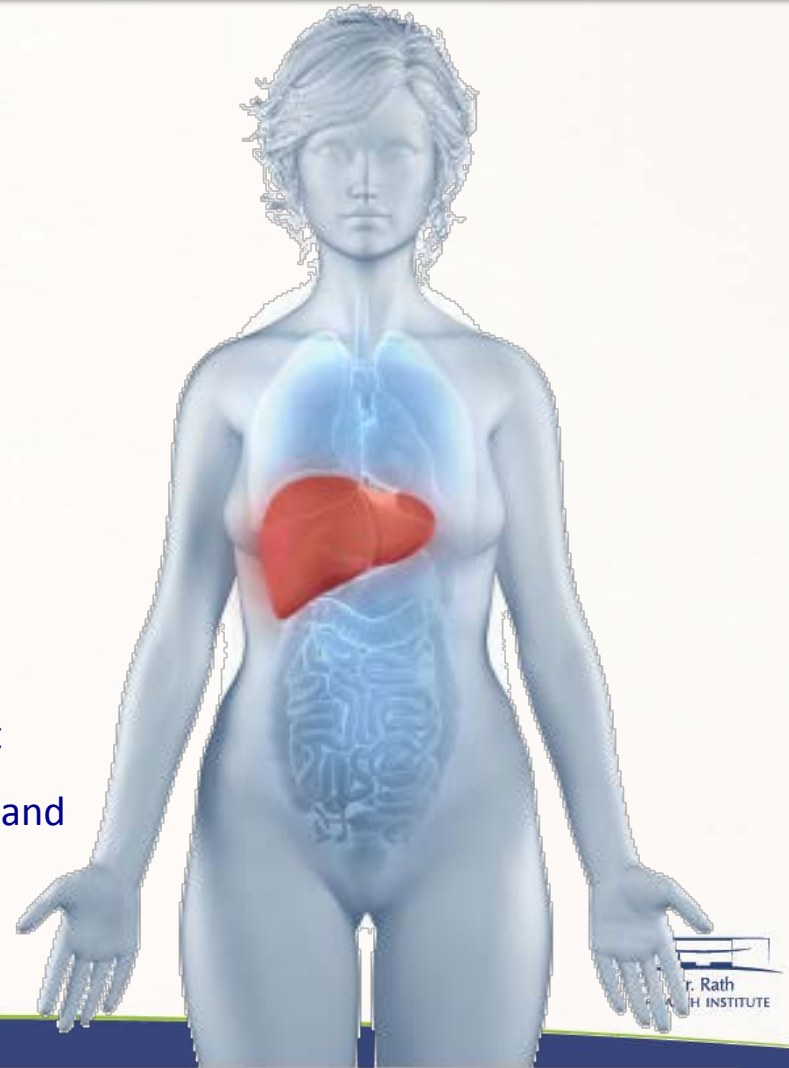
- Aggressive, spreads rapidly
- Poor prognosis, even if diagnosed early
- Symptoms: abdominal pain, weight loss, diarrhea, jaundice
- Symptoms may not appear until the cancer is advanced and surgical removal is impossible



Largest internal organ, located on the right side of the belly, above the stomach

## Functions:

- Detoxifies the blood
- Produces bile needed for the digestion of fats
- Stores some vitamins and iron
- Controls blood levels of amino acids, fats and glucose
- Breaks down food and turns it into energy
- Manufactures, breaks down and regulates hormones, including sexual hormones
- Produces enzymes and proteins, responsible for most chemical reactions in the body such as blood clotting and repair of damaged tissues



## ❑ Primary liver cancer:

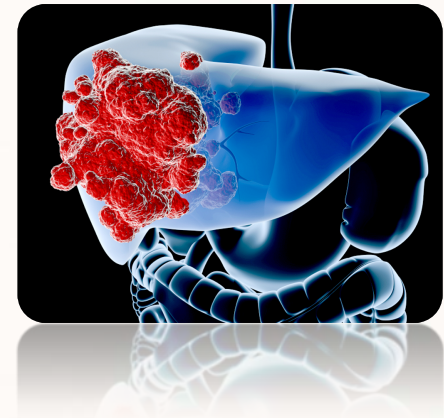
Accounts for 2% of cancers in the US, but almost 50% of all cancers in some undeveloped countries (mainly attributable to the high prevalence of hepatitis that predisposes to liver cancer)

## ❑ Symptoms:

- Significant weight loss (> 10% of body weight)
- Swelling of abdomen
- Jaundice
- Itching
- Feeling full after eating, even after a small meal

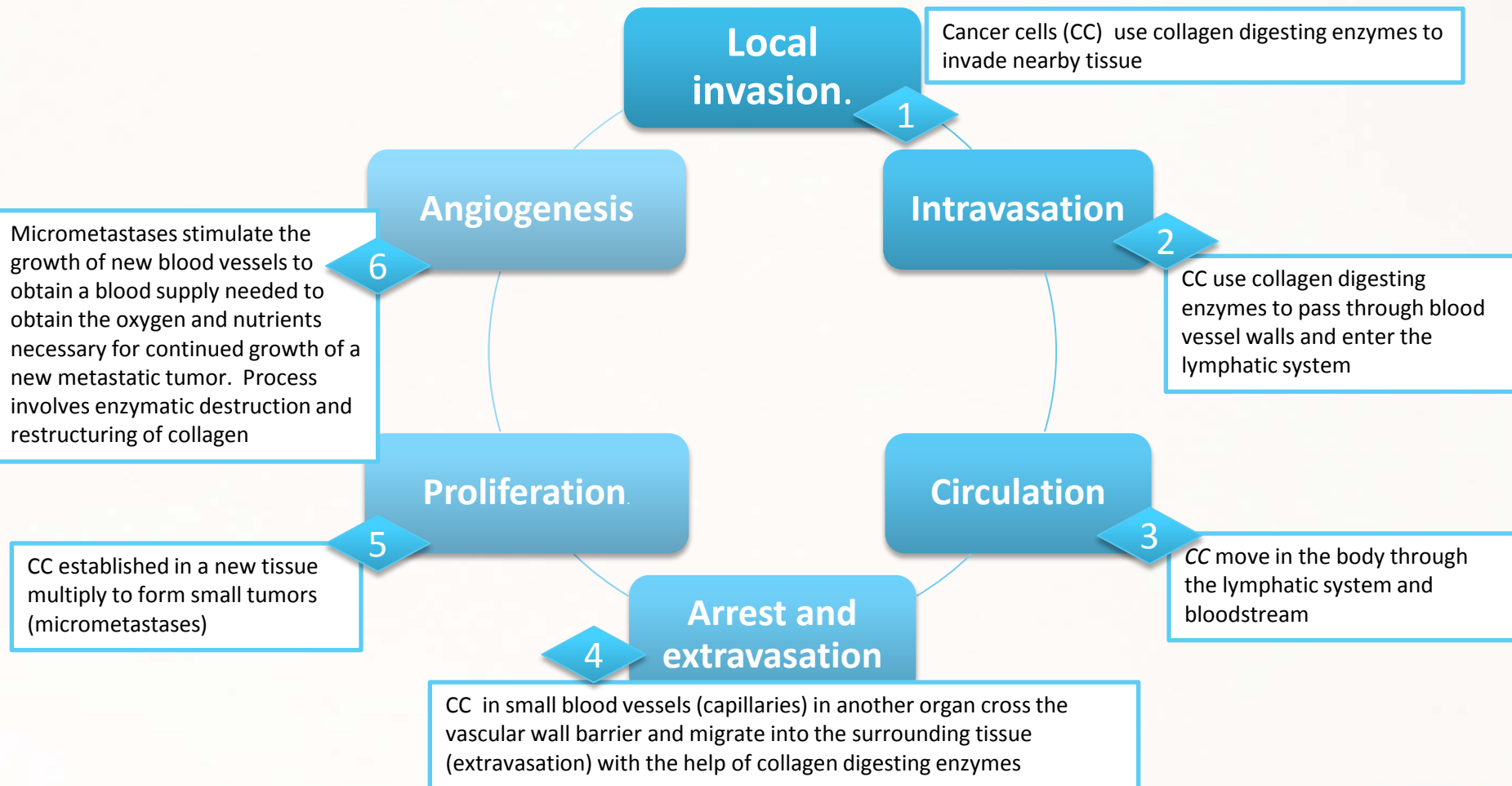
## ❑ Risk factors:

- Genetic or hereditary
- Alcohol abuse
- Chronic infection (hepatitis)
- Inherited diseases



# TUMOR INVASION AND METASTASIS

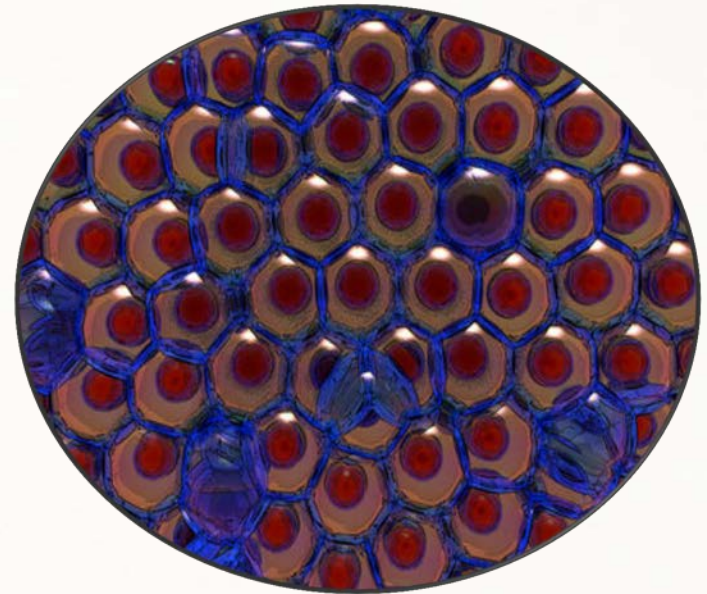
## Key steps in cancer invasion and metastasis



# TUMOR INVASION AND METASTASIS

## BREAKING EXTRACELLULAR MATRIX INTEGRITY

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



# METASTASIS

## CELLULAR MIGRATION AND EXTRACELLULAR MATRIX

### HOW CANCER CELLS INVADE OTHER ORGANS (METASTASIS)

- ✓ The collagen-dissolving mechanism is essential in tumor invasion and cancer cell migration.
- ✓ Every tumor contains a network of small blood vessels (capillaries). With the help of collagen-dissolving enzymes, cancer cells break through the blood vessel wall and enter the blood stream.
- ✓ Once in the blood stream, the cancer cells move to other organs/parts of the body via the blood flow.
- ✓ Cancer cells leave the blood stream using collagen-digesting enzymes to form metastatic (secondary) tumors.

**Tumor invasion depends on integrity of the extracellular matrix (ECM), which, when intact, acts as a barrier to block cancer cell invasion.**

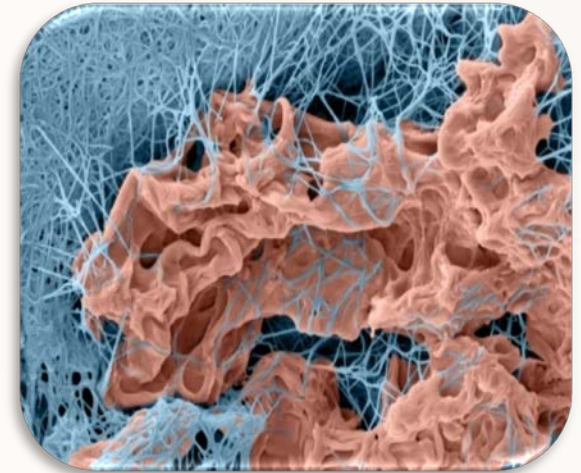
# TUMOR INVASION AND METASTASIS

## 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)

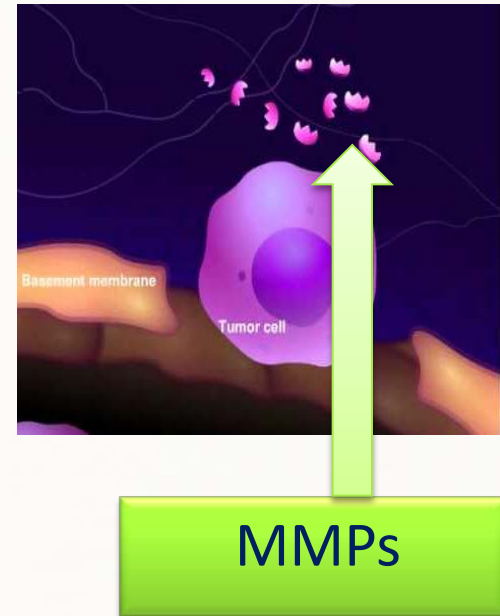
The more collagen digesting enzymes a cancer cell produces, the more aggressive the cancer is and the faster it spreads through the body.



# TUMOR INVASION AND METASTASIS

## MATRIX METALLOPROTEINASES (MMPs)

- A family of enzymes responsible for the breakdown of connective tissue proteins
- Excessive MMPs expression, especially MMP-2 and MMP-9, plays a key role in tumor cell invasion and metastasis
- Clinical studies document the association of the MMPs expression with progression of colon, pancreatic and hepatic cancer
- MMPs expression has been associated with stage IV tumors showing the highest MMPs levels





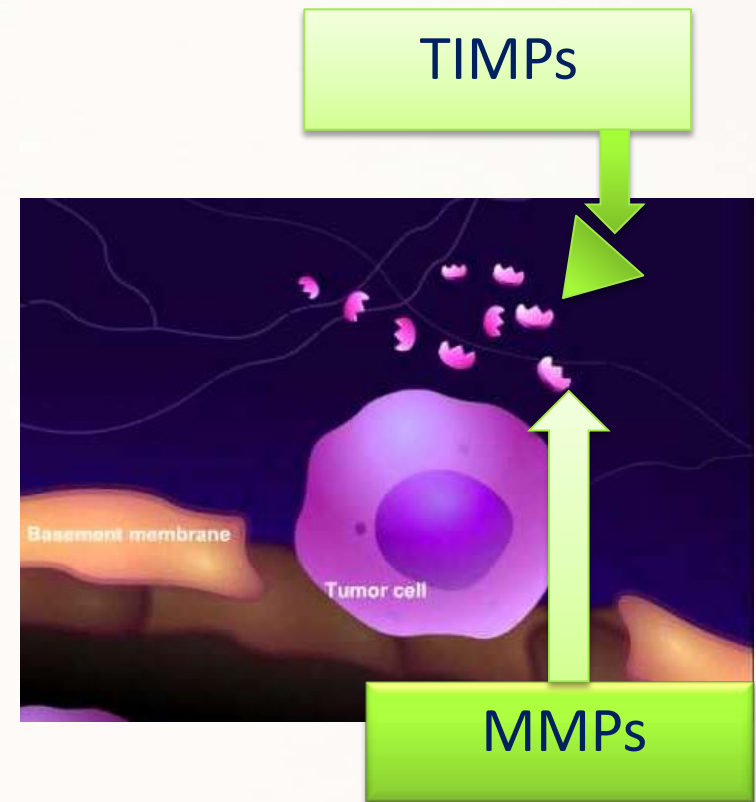
# TUMOR INVASION AND METASTASIS

## TISSUE INHIBITORS OF MATRIX METALLOPROTEINASES (TIMPs)

### Tissue inhibitors of metalloproteinases (TIMPs):

Multifunctional proteins that inhibit activities of MMPs

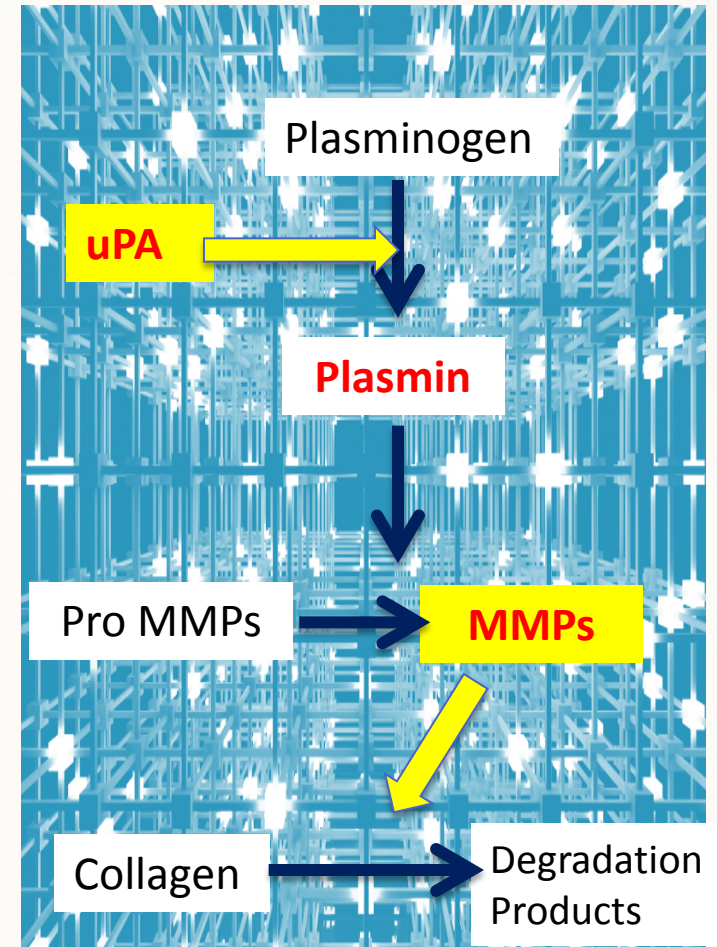
They play a role in tumor growth and metastasis



# TUMOR INVASION AND METASTASIS

## UROKINASE PLASMINOGEN ACTIVATOR (uPA)

- Components of the uPA system are overexpressed in a variety of cancer types, including colon and pancreatic cancer
- uPA converts plasminogen to plasmin, which is capable of
  - Promoting tumor growth and angiogenesis
  - Degrading the ECM
  - Activating pro MMPs
- High levels of uPA correlate with cancer progression, metastasis and poor patient prognosis




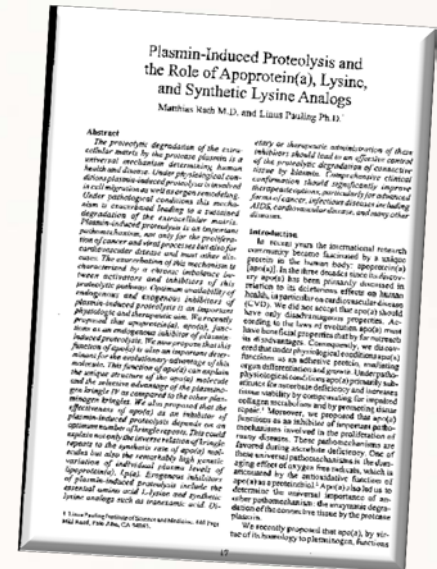
M. Rath, L. Pauling in J.Orthomol.Med . 1992



# New approach tested in our study

New approach to a natural control of cancer presented by M. Rath and L. Pauling in 1992

 This publication proposes new universal approach to halting the spread and metastasis of cancer. Its main focus is on preserving extracellular matrix integrity by controlling its proteolysis (MMPs and uPA) and at the same time optimizing its synthesis



Plasmin-Induced Proteolysis and the Role of Apoprotein(a), Lysine, and Synthetic Lysine Analogs

M. Rath, L. Pauling

Journal of Orthomolecular Medicine 1992, 7: 17-23

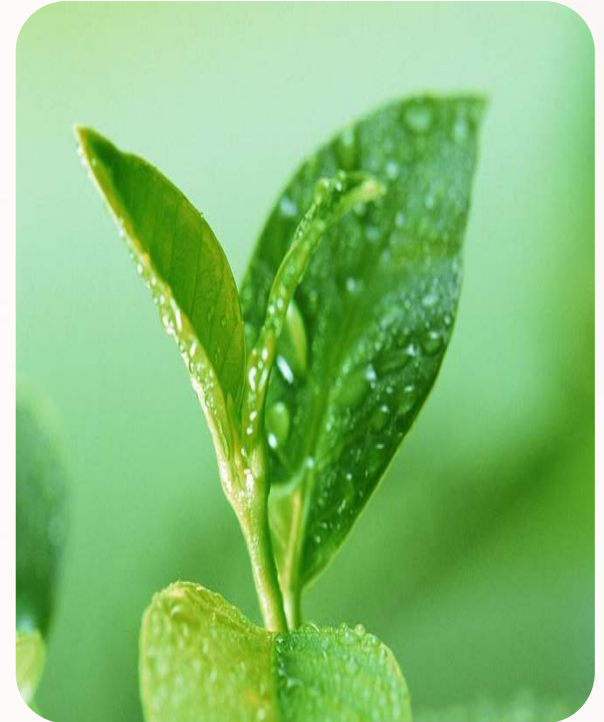




# Study objective

Investigate the effect of a nutrient mixture containing natural inhibitors of ECM digestion and activators of its production on the activities of uPA, MMP-2, MMP-9 and TIMPs in human colon, pancreatic and hepatic carcinoma cell lines.

Earlier studies with this nutrient mixture have shown its effectiveness in inhibiting metastasis and MMPs activity in various types of cancers.



For earlier studies visit:  
[www.drrathresearch.org](http://www.drrathresearch.org)



# Composition of nutrient mixture (NM) used in the study

The nutrient mixture (NM) was composed of the following ingredients in the relative amounts indicated:

- Vitamin C (as ascorbic acid, Mg and Ca ascorbates and ascorbyl palmitate ), 700 mg
- 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 $\mu$ g
- Copper, 2 mg
- Manganese, 1 mg

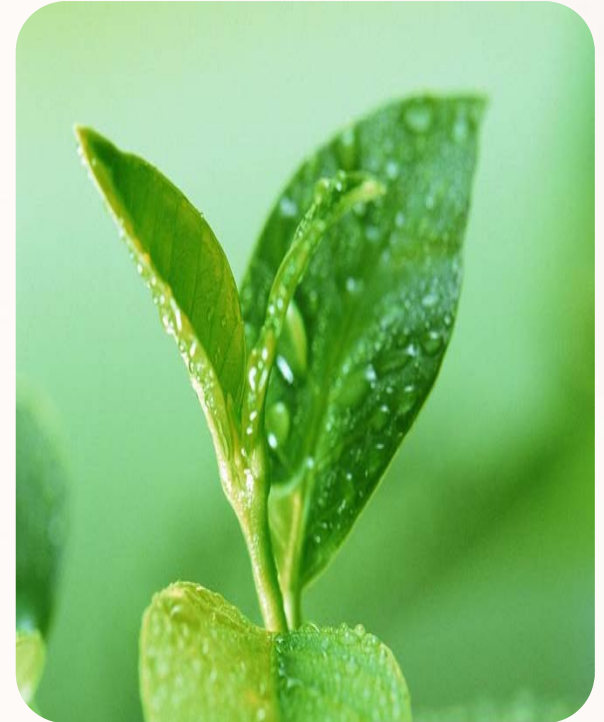


# In Vitro tests

Human colon, pancreatic and liver (hepatocellular carcinoma) cancer cell lines were grown in tissue culture media.

Cells were treated in triplicate with different concentrations of the nutrient mixture:

- For uPA and TIMP activity tests cells were treated with NM at 0, 50, 100, 250, 500, 1000  $\mu\text{g}/\text{ml}$
- For MMP analysis cells were treated with NM at 0, 10, 50, 100, 500, 1000  $\mu\text{g}/\text{ml}$

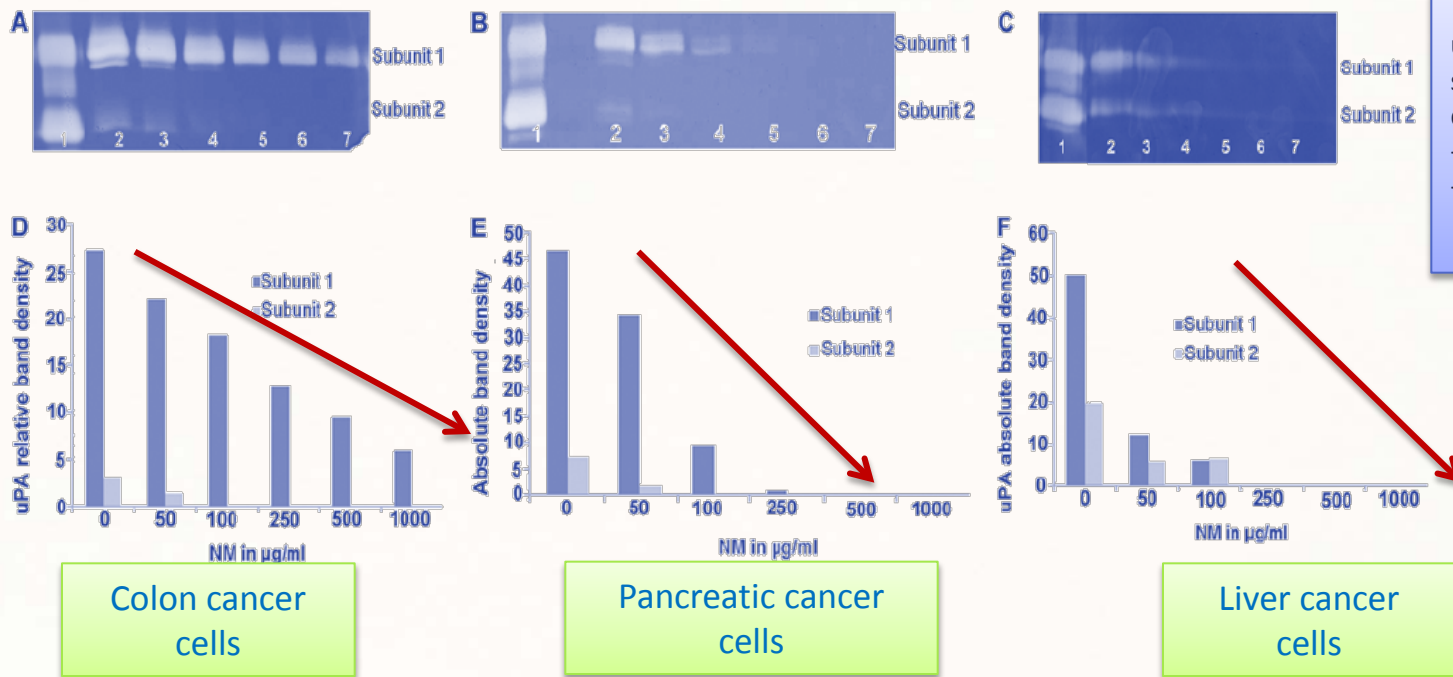




- **The effects of NM on the following enzymatic activities in human colorectal, pancreatic and liver cancer cells**
  1. uPA activity
  2. MMP-2 and MMP-9 secretion
  3. TIMP activity
- **Correlation between uPA, TIMP-2 and MMP expression levels**



# NM inhibits uPA activity in human colorectal, pancreatic and liver carcinoma cell lines



u-PA consists of two subunits, joined by a S-S-disulphide bridge

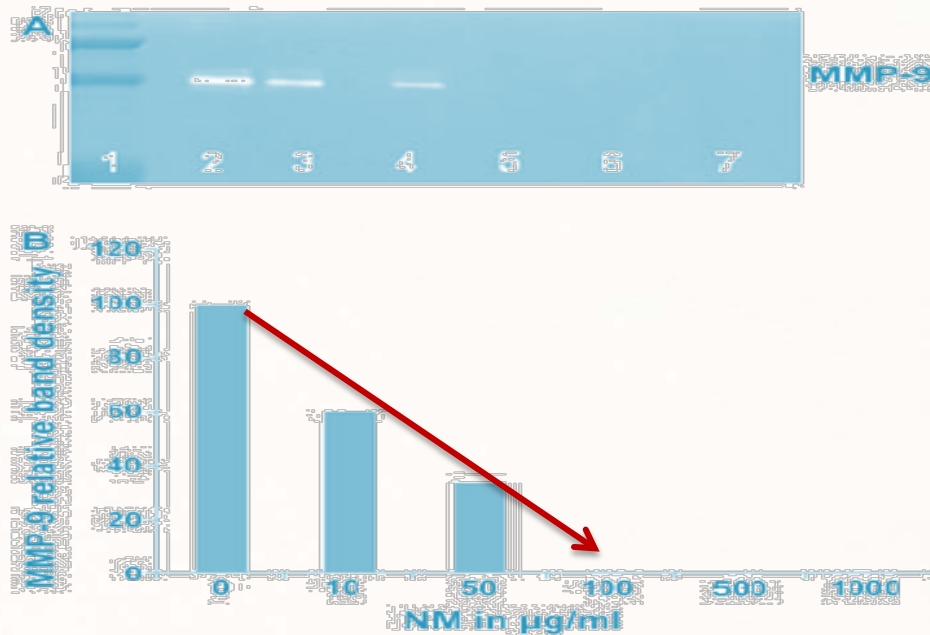
- Subunit 1(35KDa)
- Subunit 2 (33 Kda)

NM inhibited uPA activity in colorectal, pancreatic and liver carcinoma in a dose-dependent manner.





# NM inhibits secretion of MMP-2 and MMP-9 in colon cancer cells



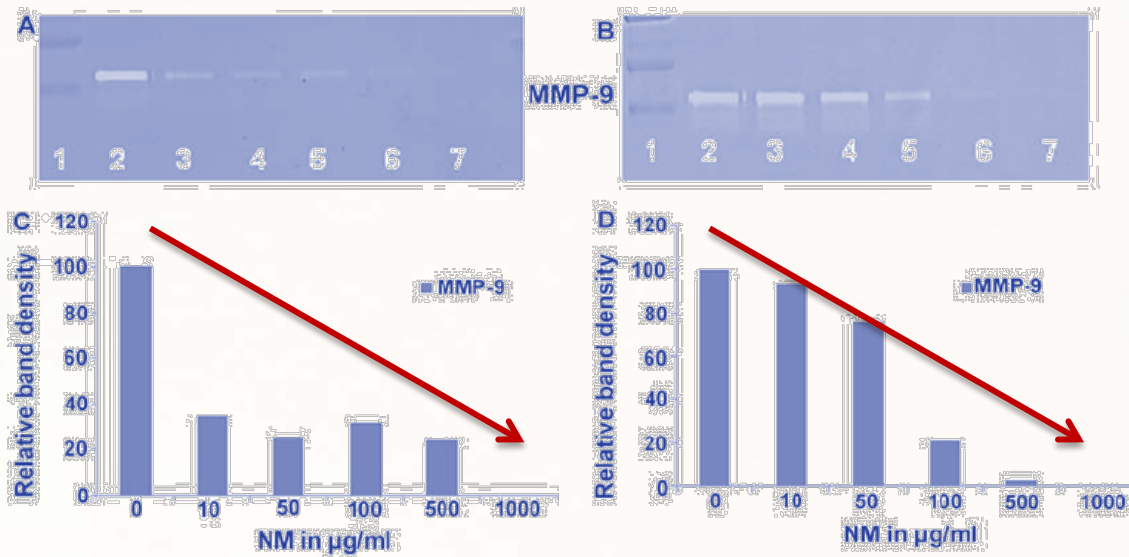
Colon cancer cell line (HCT-116) does not express MMP2. These cancer cells produce MMP-9, which was inhibited by NM in a dose-dependent manner with total (100%) inhibition of MMP-9 at 100  $\mu\text{g/ml}$ .



# NM inhibits MMP-9 secretion in pancreatic cancer cells

## Pancreatic cancer cell line (MIA PaCa-2)

- These cancer cells do not secrete MMP-2
- These cancer cells secrete MMP-9, which is enhanced by the exposure to PMA (phorbol 12-myristate 13-acetate)
- NM inhibited MMP-9 secretion by untreated pancreatic cancer cells at 1000  $\mu\text{g/ml}$  and also PMA-treated cells at 1000  $\mu\text{g/ml}$



Pancreatic cancer cells

Pancreatic cancer cells exposed to PMA

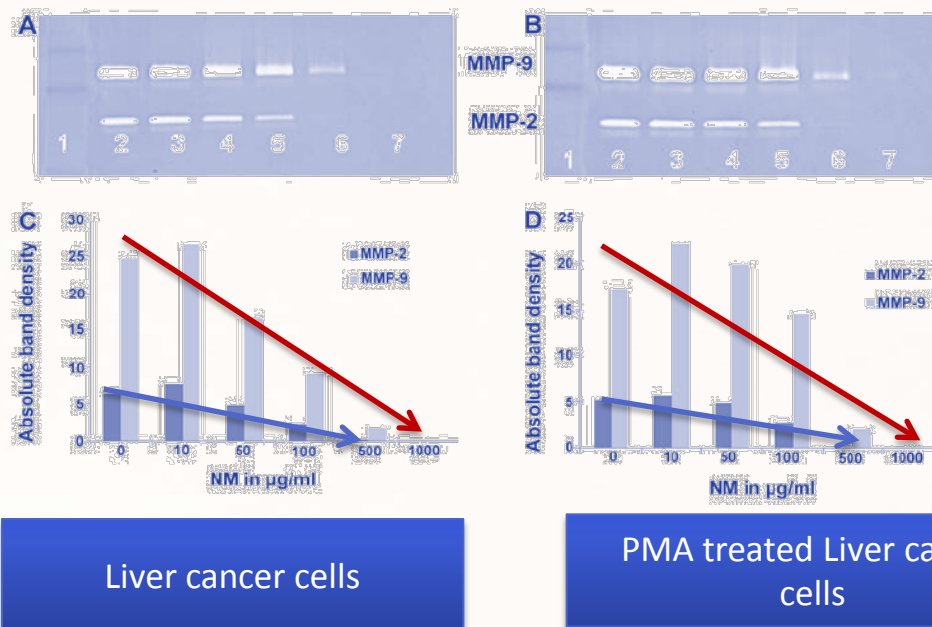
PMA =  
a potent tumor  
promotor and  
inducer of MMPs



# NM inhibits MMP-2 and MMP-9 secretion by liver cancer cells

## Liver cancer cell line (HCC cell line SK-Hep-1)

- These cells secrete MMP-9 and MMP-2 enzymes without and with PMA stimulation
- NM inhibits the secretion of these enzymes in a dose-dependent manner with total inhibition of MMP-9 at 1000  $\mu\text{g/ml}$  and MMP-2 at 500  $\mu\text{g/ml}$ . In PMA-treated cells the MMP-9 and MMP-2 secretion was 100% inhibited at these concentrations, respectively.

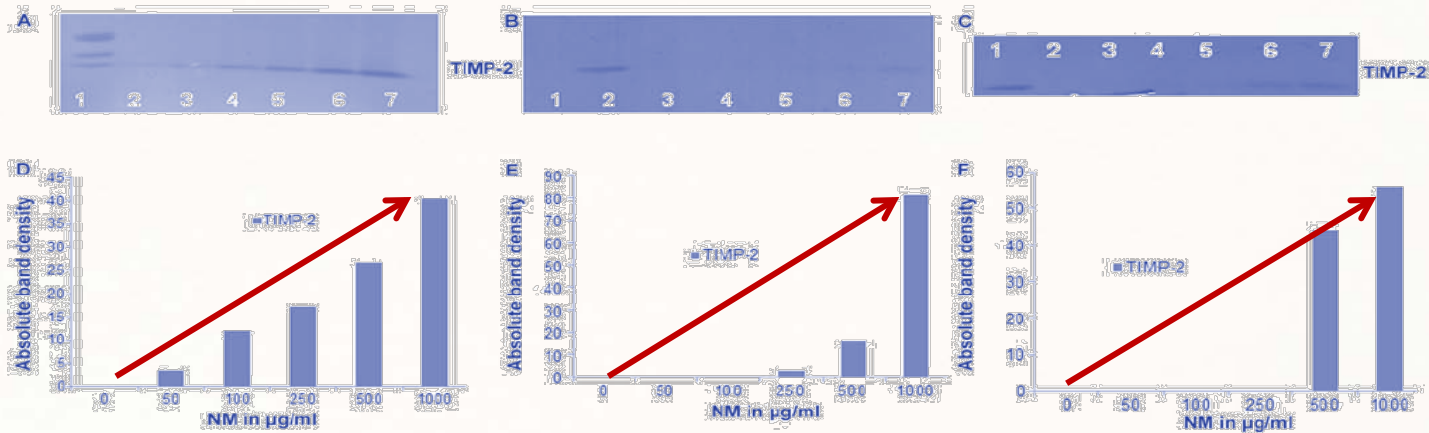




# NM stimulates TIMP-2 activity in colon, pancreatic and liver cancer cells

TIMP-2 activity increased in all cancer cell lines after exposure to NM in a dose-dependent manner. Minimum TIMP-2 activity was expressed at NM of 50  $\mu\text{g/ml}$  (colon cancer) and maximum at 1000  $\mu\text{g/ml}$  NM all tested cancer cells


Effect of NM on uPA activity in human colorectal, pancreatic and liver carcinoma cell lines



Colon cancer cells

Pancreatic cancer cells

Liver cancer cells



# Inhibition of uPA and MMPs by NM correlates with higher expression of TIMP-2 in colon, pancreatic and liver cancer cell lines

We evaluated whether activity of uPA, MMPs and TIMP-2 in all tested cells exposed to different NM concentrations have some relation to each other by calculating their correlation coefficients.

- A positive correlation was found between uPA and MMP-9 expression levels of NM treated colon, pancreatic and liver carcinoma, which means that **NM was effective in simultaneously inhibiting MMP-9 and uPA in these cancer cell types**
- Negative correlations were found between the expression of MMP-9 and TIMP-2 in all three cancer cell lines treated with NM. This means that **stimulation of TIMP-2 by the exposure of cells to NM was accompanied by a decrease in MMP-9 activity**
- Negative correlations were found between expression levels of TIMP-2 and uPA in all three cell lines treated with NM which means that **stimulation of TIMP-2 by the exposure of cells to NM was accompanied by a decrease in uPA activity**

# SUMMARY OF THE RESULTS



1. Exposure of gastrointestinal cancer cells to the specific mixture of nutrients containing natural inhibitors of extracellular matrix proteolysis resulted in :
  - Inhibition of uPA secretion in colon, pancreatic and liver cell lines
  - Decrease in MMP9 and MMP2 secretions
  - Increase in TIMP-2 secretion by all these cell lines.
2. A positive correlation was found between the secretion of uPA and MMP-9.
3. A negative correlation was found between uPA and TIMP-2 and between MMP-2 and TIMP-2 secretion by NM treatment of colon, pancreatic and liver cancer cell lines.

# IMPLICATIONS OF THESE FINDINGS



The nutrient mixture tested in this study demonstrated a potent anticancer activity by targeting important mechanisms responsible for aggressive spread and metastasis of colon, pancreas and liver cancers, which is enzymatic degradation of extracellular matrix.

This nutrient mixture does not have toxic effects.

**Hence, dietary intake of the mixture of phytonutrients should be considered in developing safe and effective approaches to effective control GI cancers.**