



# Antineoplastic Activity of a Novel Nutrient Mixture on Malignant Retinoblastoma Cell Line Y79

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**Presented at:** International Congress of Toxicology, Montreal, Canada, July 15-19, 2007

**Published in:** International Congress of Toxicology, Carcinogenesis, July 2007, Abstract #PW2.042, page 84

## **Abstract**

### **Introduction:**

Retinoblastoma (Rb), a malignant tumor of the retina affecting children under the age of six, metastasizes to extraocular organs including bone, lung and brain. Currently there is no effective treatment. We previously demonstrated that a unique non-toxic nutrient mixture (NM) consisting of lysine, proline, ascorbic acid and green tea extract has an antineoplastic activity against a number of cancer cell lines both in vivo and in vitro.

### **Objective:**

We investigated the antineoplastic activity of NM on human malignant retinoblastoma cell line Y79 evaluating proliferation, MMP expression and invasion through Matrigel.

### **Materials and Methods:**

Human retinoblastoma cells (ATCC) were grown in RPMI-1640 medium with 20% FBS and antibiotics and treated with NM at 0, 10, 50, 100, 500 and 1000  $\mu\text{g}/\text{ml}$  in triplicate at each dose. Cell proliferation was assessed by counting cells stained with trypan blue, invasion through Matrigel, and MMP activity by gelatinase zymography. Cells were also treated with PMA to induce MMP-9 activity

### **Results:**

Rb cells exhibited 25% toxicity to 10-100  $\mu\text{g}/\text{ml}$  NM and profound toxicity at 500 and 1000  $\mu\text{g}/\text{ml}$  NM. Zymography demonstrated production of only MMP-2. NM at 10-100  $\mu\text{g}/\text{ml}$  had no effect on MMP-2, but completely blocked it at 500  $\mu\text{g}/\text{ml}$ . PMA treatment did not induce MMP-9. Interestingly, Rb cells were not invasive through Matrigel.

### **Conclusion:**

In conclusion, these results suggest that NM may have therapeutic potential in the treatment of retinoblastoma.

**Comment:**

Currently there is no effective treatment for retinoblastoma (Rb), a malignant tumor of the retina which affects children under the age of six. Rb metastasizes to extraocular organs including bone, lung and brain. We investigated the in vitro antineoplastic activity of a non-toxic nutrient mixture (NM) consisting of lysine, proline, ascorbic acid and green tea extract that has shown anticancer activity against a number of cancer cell lines on human malignant retinoblastoma cell line Y79. Rb cells exhibited 25% toxicity to 10-100 µg/ml NM and profound toxicity at 500 and 1000 µg/ml NM. Zymography demonstrated production of only MMP-2. NM at 10-100 µg/ml had no effect on MMP-2, but completely blocked it at 500 µg/ml. PMA treatment did not induce MMP-9. Interestingly, Rb cells were not invasive through Matrigel. These results are significant as they suggest NM may have therapeutic potential in the treatment of retinoblastoma.

**ANTINEOPLASTIC ACTIVITY OF A NOVEL NUTRIENT MIXTURE ON MALIGNANT RETINOBLASTOMA CELL LINE Y79**

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**Introduction:**  
 Retinoblastoma, a malignant tumor of the retina affecting children under the age of six, metastasizes to extraocular organs including bone, lung and brain. Currently there is no effective treatment. We previously demonstrated that a unique non-toxic nutrient mixture (NM) consisting of lysine, proline, ascorbic acid and green tea extract has an antineoplastic activity against a number of cancer cell lines both in vivo and in vitro.

**Methods:**  
 1. We investigated the antineoplastic activity of NM on human malignant retinoblastoma cell line Y79 evaluating proliferation, MMP expression and invasion through Matrigel.

2. Human retinoblastoma Y79 cells (ATCC) were grown in RPMI-1640 medium with 20% FBS and antibiotics and treated with NM at 0, 10, 50, 100, 500 and 1000 µg/ml in triplicate at each dose.

3. Cell proliferation was assessed by counting cells stained with trypan blue, invasion through Matrigel, and MMP activity by gelatinase zymography. Cells were also treated with PMA to induce MMP-9 activity.

**Composition of the Nutrient Mixture (NM)**

Nutrient	Proportion
Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbates)	710 mg
L-Lysine	1000 mg
L-Proline	250 mg
L-Arginine	500 mg
N-Acetyl Cysteine	200 mg
Standardized Green Tea Extract (80% polyphenols)	1000 mg
Selenium	30 µg
Copper	2 mg
Manganese	1 mg

**Results:**  
 1. Retinoblastoma cells exhibited 25% toxicity to treatment with 10-100 µg/ml NM, and profound toxicity at 500 and 1000 µg/ml NM, as shown in Figure 1.

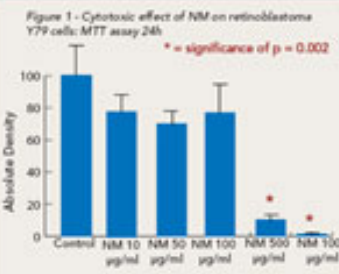
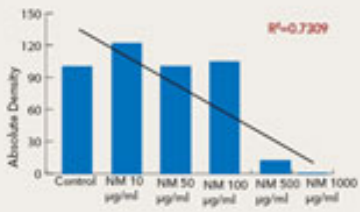


Figure 2B - Effect of NM on retinoblastoma Y79 MMP-2 secretion: densitometry analysis



1. Interestingly, retinoblastoma Y79 cells were not invasive through Matrigel.

2. Zymography demonstrated production of only MMP-2 (Figures 2A, 2B) by retinoblastoma Y79 cells. NM at 10-100 µg/ml had no effect on MMP-2 secretion, but completely blocked it at 500 µg/ml. PMA treatment did not induce MMP-9 secretion.

Figure 2A - Effect of NM on retinoblastoma cell MMP secretion: gel zymography



Legend: 1 - Markers, 2-Control, 3-7 NM 10, 50, 100, 500, 1000 µg/ml

**Conclusion:**  
 These results suggest that NM may have therapeutic potential in the treatment of retinoblastoma.