



# Suppression of Growth In Vivo and In Vitro of Murine B16FO Melanoma Cells by a Novel Nutrient Mixture

M.W. Roomi, V. Ivanov, A. Niedzwiecki and M. Rath

Dr. Rath Research Institute, Oncology Division, 1260 Memorex Drive, Santa Clara, CA 95050

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## **Introduction:**

A novel nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract has exhibited anti-tumor activity *in vivo* and *in vitro*. In this study we examined the effect of NM on melanogenesis *in vivo* and *in vitro* using B16-F0 melanoma cell line. In advanced stages, highly metastatic melanoma is resistant to existing therapies.

## **Objective:**

We investigated the effect of NM on murine B16FO melanoma cells *in vitro* evaluating viability, MMP secretion, invasion, morphology and apoptosis. *In vivo* studies were carried out in athymic nude mice bearing B16-F0 xenografts.

## **Methods:**

Athymic nude male mice, 5-6 weeks old, were inoculated with  $1 \times 10^6$  B16-FO melanoma cells (ATCC) subcutaneously and randomly divided into two groups; group A was fed a regular diet and group B a regular diet supplemented with 0.5% NM. Four weeks later, the mice were sacrificed and their tumors were excised, weighed and processed for histology. We also tested the effect of NM *in vitro*, measuring cell proliferation by MTT assay, invasion through Matrigel, secretion of MMPs by gelatinase zymography, cell morphology by H&E staining and apoptosis using live green caspase detection kit (Molecular Probes).

## **Results:**

NM inhibited the growth of B16-FO melanoma cells *in vivo* by 50%. Lesions, both in control and test groups were composed of cords and nests of large, irregularly round, pigmented cells consistent with a malignant melanoma. *In vitro*, NM was not toxic to the melanoma cells at 100 mg/ml concentration, but exhibited 50% toxicity over the control at 500 and 1000 mg/ml. H&E did not indicate any morphological changes up to 100 mg/

ml. B16-F0 melanoma cells demonstrated no MMP secretion nor invasion through Matrigel. NM induced slight apoptosis at 100 mg/ml, moderate at 500 and extensive at 1000 mg/ml concentration.

### Conclusion:

Taken together these results suggest that NM has many attractive features as a new anti-tumor agent.

**Comment:** Melanoma is a very serious and highly metastatic form of skin cancer, which causes the most skin cancer-related deaths. In its advanced stages melanoma is resistant to existing therapies. We investigated the effect of a unique nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract on murine B16F0 melanoma cells in vitro and also in vivo by injecting these melanoma cells under the skin of nude mice. After 4 weeks of supplementation with NM growth of melanoma tumors in mice was inhibited by 50%. Melanoma cells exposed to 500 and 1000 mg/ml NM concentration in vitro, exhibited 50% toxicity over the control. At these concentrations a moderate and extensive apoptosis (natural cell death) was observed respectively. These results are significant as they suggest NM as a therapeutic agent for melanoma.

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M.W. Roomi, V. Ivanov, A. Niedzwiecki, M. Rath  
Dr. Rath Research Institute, Santa Clara, CA 95050

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**Introduction**  
In advance stages, highly metastatic melanoma is resistant to existing therapies. A novel nutrient mixture (NM) containing lysine, proline, ascorbic acid, and green tea extract has exhibited anti-tumor activity in vivo and in vitro. In this study we examined the effect of NM on melanogenesis in vivo and in vitro using B16F0 melanoma cell line.

**Objective**  
We investigated the effect of NM on murine B16F0 melanoma cells in vitro evaluating viability, MMP secretion, invasion, morphology and apoptosis. In vivo studies were carried out in athymic nude mice bearing B16F0 xenografts.

**Methods**  
**in vivo**  
1. Athymic nude male mice, 5-6 weeks old, were inoculated with  $1 \times 10^6$  B16-F0 melanoma cells (ATCC) subcutaneously.  
2. The mice were randomly divided into two groups: Group A was fed a regular diet and Group B a regular diet supplemented with 0.5% NM.  
3. Four weeks later the mice were sacrificed and their tumors were excised, weighed and processed for histology.

**in vitro**  
1. B16F0 cells were cultured in the appropriate medium and in the presence of NM at 0, 10, 50, 100, 500 and 1000 µg/ml concentration in triplicate at each concentration.  
2. Cell proliferation was measured by MTT assay, invasion through Matrigel, MMPs by gelatinase zymography, morphology by H&E staining Apoptosis was assayed using live green caspase detection kit (Molecular Probes).

**Composition of Nutrient Mixture (NM)**

Nutrient	Key Source
Water C, ascorbic acid and as My, Ca and potassium ascorbate	750 mg
Glutamine	1000 mg
L-Proline	750 mg
L-Arginine	500 mg
N-Acetyl Cysteine	200 mg
Standardized Green Tea Extract (95% polyphenols)	1000 mg
Selenium	30 µg
Copper	2 mg
Manganese	1 mg

**Results**

1. NM inhibited the growth of B16F0 melanoma tumor xenografts in athymic nude mice by 52% (Figure 1). Lesions both in control and supplemented groups were composed of cords and nests of large, irregularly round, pigmented cells consistent with a malignant melanoma (Figures 2A - D).

2. NM was not toxic at 100 µg/ml. However, it exhibited 44% toxicity over the control at 500 µg/ml and 1000 µg/ml, as shown in Figure 3.

3. B16F0 melanoma cells exposed to different concentrations of NM did not show any morphological changes at or below 1000 µg/ml NM by Hematoxylin & Eosin staining, as shown in Figure 4.

4. B16F0 melanoma cells did not demonstrate any MMP secretion by zymography or invasion through Matrigel.

5. NM induced slight apoptosis of B16F0 melanoma cells at 100 µg/ml, moderate at 500 µg/ml, and extensive at 1000 µg/ml NM.

**Figure 1 - Effect of NM on growth of B16F0 melanoma tumors**

Group	Tumor Weight (g)
Control	~1.1
NM 0.5%	~0.5

**Figure 2 - Histopathology of B16F0 Tumors**

2A - Control (H&E) 2B - Control (200x) 2C - NM 0.5% (H&E) 2D - NM 0.5% (200x)

**Figure 3 - Effect of NM on B16-F0 melanoma cell proliferation**

Concentration (µg/ml)	Cell Proliferation (% of Control)
Control	100
100	~100
50	~100
1000	~50
500	~50

**Figure 4 - Effect of NM on morphology of B16F0 melanoma cells (H&E staining)**

4A - Control 4B - NM 100 µg/ml 4C - NM 500 µg/ml 4D - NM 1000 µg/ml

**Figure 5 - Effect of NM on B16-F0 melanoma cell morphology**

5A - Control 5B - NM 100 µg/ml 5C - NM 500 µg/ml 5D - NM 1000 µg/ml

Positive control Caspase3 8µg/ml

**Conclusion**  
Taken together these results suggest that NM has many attractive features as a new antitumor agent.