Suppression of Growth, *in Vivo* and *in Vitro*, of Murine B16FO Melanoma Cells by a Novel Nutrient Mixture

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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 B16FO melanoma cell line.

Objective

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

Methods

In Vivo

1. Athymic nude male mice, 5-6 weeks old, were inoculated with 1 x 10^6 B16-FO 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. B16FO 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 (Molecualr Probes).

Composition of Nutrient Mixture (NM)

Nutrient	Per Stock Solutions
Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbate)	710 mg
L-Lysine	1000 mg
L-Proline	750 mg
L-Arginine	500 mg
N-Acetyl Cysteine	200 mg
Standardized Green Tea Extract (80% polyphenol)	1000 mg
Selenium	30 µg
Copper	2 mg
Manganese	1 mg

Results

1. NM inhibited the growth of B16FO melanoma tumor xenografts in athymic nude mice by 53% (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).

Figure 1 - Effect of NM on growth of B16FO melanoma tumors

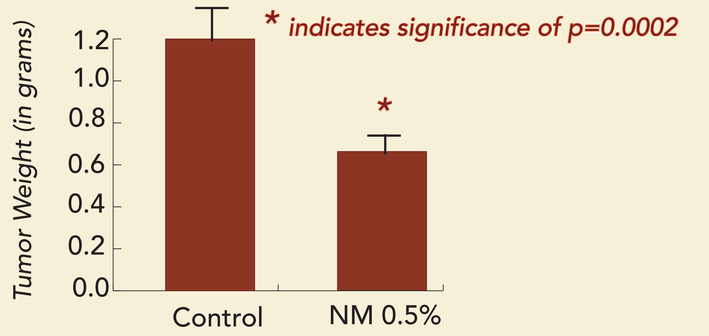
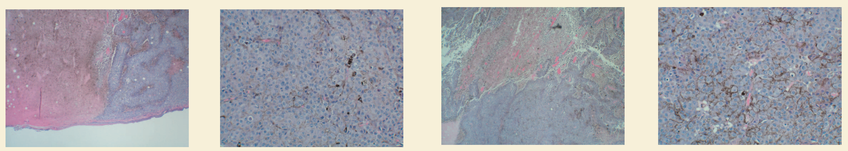


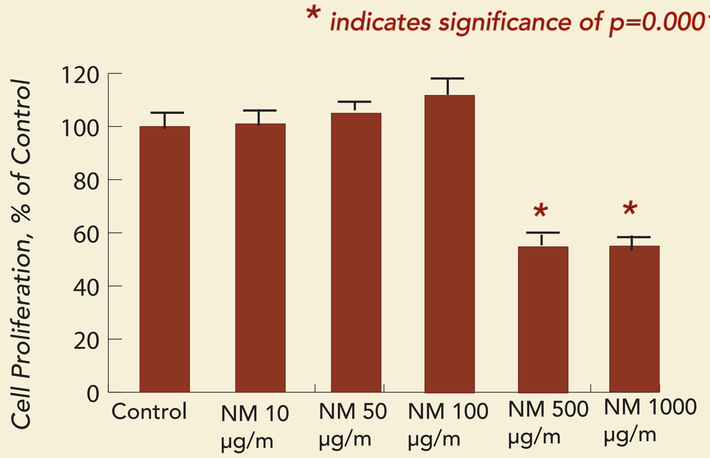
Figure 2- Histopathology of B16FO Tumors



2A - Control (40x) 2B - Control (200x) 2C - NM 0.5% (40x) 2D - NM 0.5% (200x)

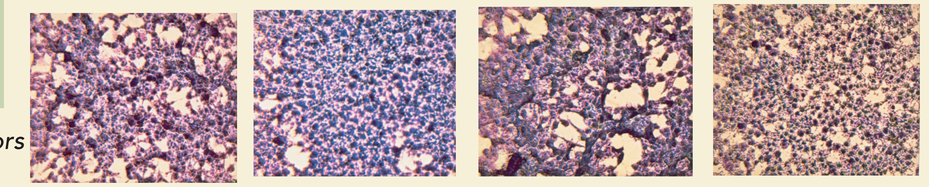
2. NM was not toxic at 100 µg/ml. However, it exhibited 44% toxicity over the control at 500 µg/ml and 1000 µg/ml, as shown in Figure 3.

Figure 3 - Effect of NM on B16-FO melanoma cell proliferation



3. B16FO 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.

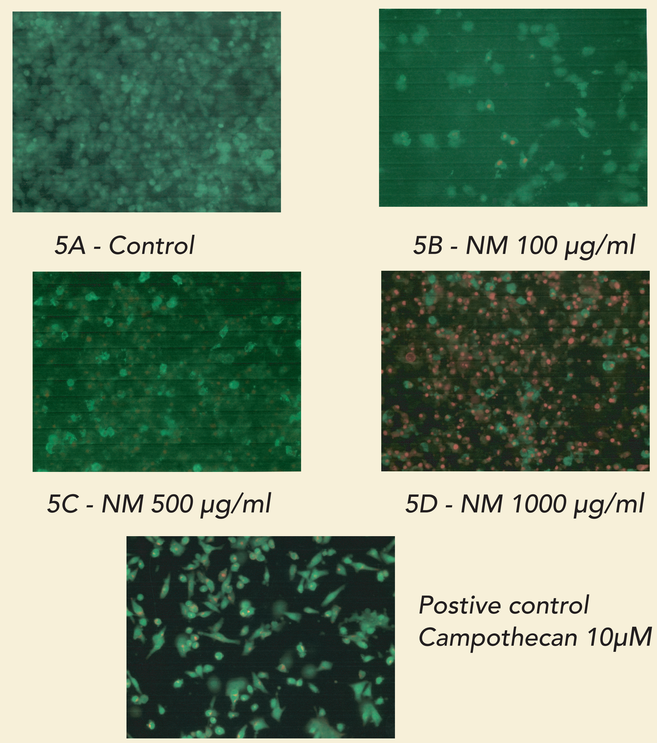
Figure 4 - Effect of NM on morphology of B16FO melanoma cells: H&E staining



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

4. B16FO melanoma cells did not demonstrate any MMP secretion by zymography or invasion through Matrigel.

5. NM induced slight apoptosis of B16FO melanoma cells at 100 µg/ml, moderate at 500 µg/ml, and extensive at 1000 µg/ml NM.



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

Postive control Campothecan 10µM

Conclusion

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