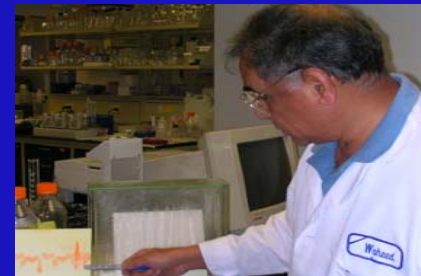


A Nutrient Mixture Induces Apoptosis in Human Renal Cell Carcinoma 786-0 and Human Melanoma Cell Line A2058

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Introduction

Renal cell carcinoma (RCC) is erratic and unpredictable even when diagnosed. Incidence of RCC is higher among African Americans than among Caucasians. The male to female ratio for RCC is 2:1. At diagnosis, five-year survival is limited to 60% in RCC patients.

Melanoma, a very serious form of skin cancer, is a relatively rare cancer. However, its incidence rate in the U.S. has been increasing steadily, and in 2003, it was the sixth most common cancer in the U.S. Though often curable in its early stages, melanoma may metastasize to other areas of the body.

Introduction

A novel nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract has shown a wide range of pharmacological properties, including a broad spectrum of antitumor activity against a number of human cancer cell lines.

We investigated whether the underlying antitumor effect of NM was due to apoptosis in human RCC and melanoma, studying its effect on caspases, proliferation and morphology. In addition, we investigated its effect on MMP-2 and MMP-9 secretion and Matrigel invasion of these cells.

Composition of Nutrient Mixture (NM)

| Nutrient | Molar Concentration (in 100 µg/ml solution) |
|--------------------------|--|
| Vitamin C | 90 µM |
| L-Lysine | 110 µM |
| L-Proline | 110 µM |
| L-Arginine | 50 µM |
| N-Acetyl Cysteine | 25 µM |
| Green Tea Extract | EGCG 15 µM |
| Selenium | 8.5 µM |
| Copper | 700 nM |
| Manganese | 400 nM |

Methods

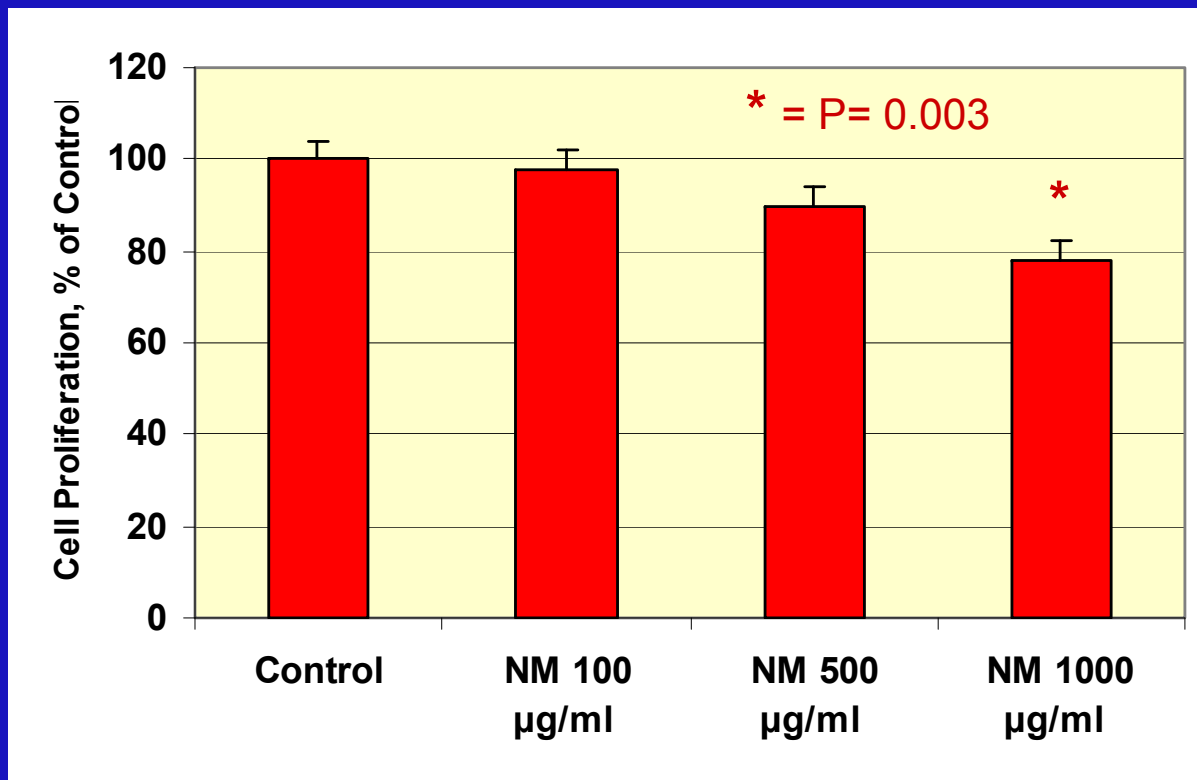
Human RCC 786-0 and human melanoma cells A2058 (ATCC) were cultured in RPMI and DMEM media respectively, supplemented with 10% FBS and antibiotics. The cells were then challenged with NM at 0, 50, 100, 500 and 1000 $\mu\text{g/ml}$ concentration in triplicate at each concentration.

- Cell toxicity was evaluated by MTT assay.
- Apoptosis was determined by Live Green Caspase Detection Kit
- Morphology was assessed by H&E staining
- MMP-2 and MMP-9 secretion by gelatinase zymography
- Invasion through Matrigel

Effect of NM on RCC 786-0 Toxicity

MTT Assay – 24h

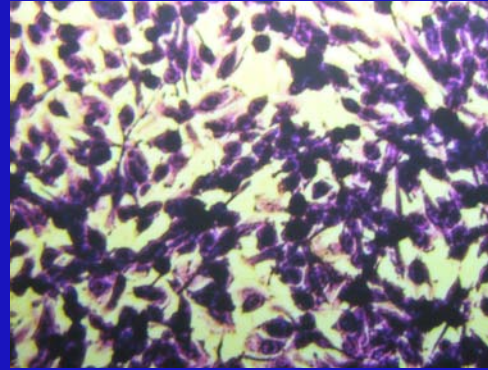
NM showed slight insignificant toxicity to RCC 786-0 cells at 100 $\mu\text{g/ml}$ NM, 10% at 500 $\mu\text{g/ml}$ and 22% at 1000 $\mu\text{g/ml}$.



Effect of NM on RCC 786-0 Morphology

H&E stain

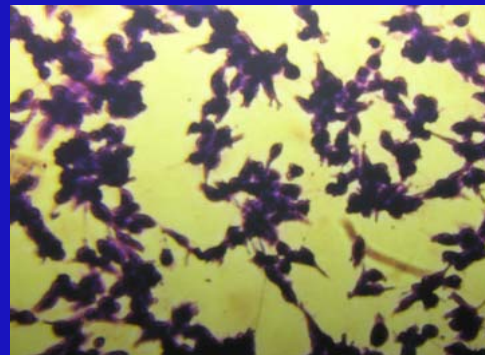
Though NM was not toxic to RCC 786-0 cells, cells exposed to NM at 500 and 1000 $\mu\text{g/ml}$ showed obvious apoptosis by H&E stain. Apoptotic cells showed shrinkage with darkly stained and condensed nuclei and strong acidophilic cytoplasm.



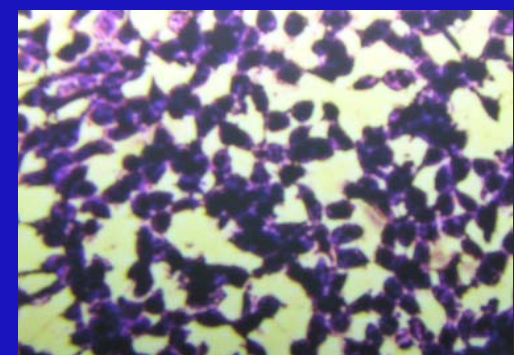
Control



NM 100 $\mu\text{g/ml}$



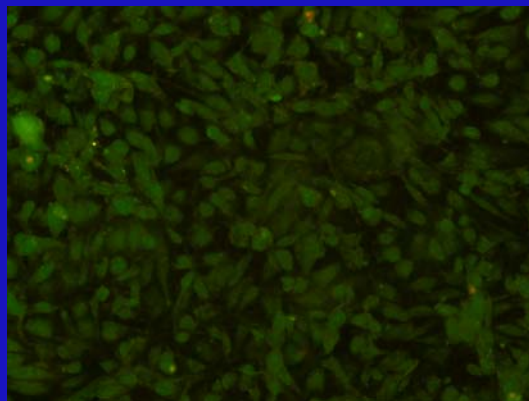
NM 500 $\mu\text{g/ml}$



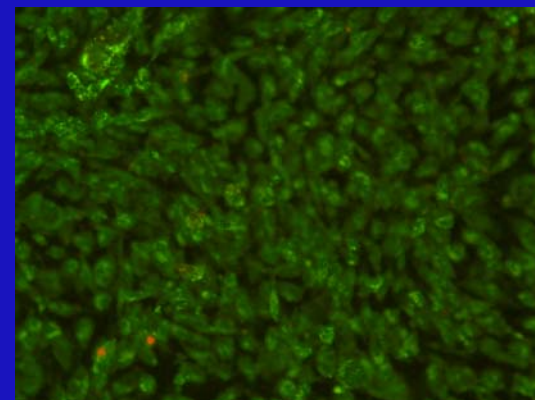
NM 1000 $\mu\text{g/ml}$

Effect of NM on RCC 786-0 Apoptosis: Live green caspase detection kit

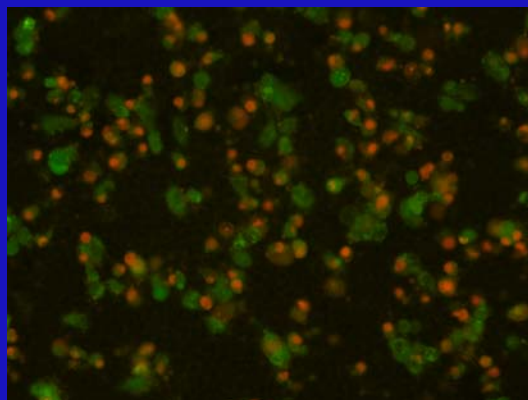
Using the Live Green Caspases Detection Kit, a significant number of early and late apoptotic cells were evident when RCC 786-0 cells were exposed to 500 and 1000 $\mu\text{g/ml}$ NM.



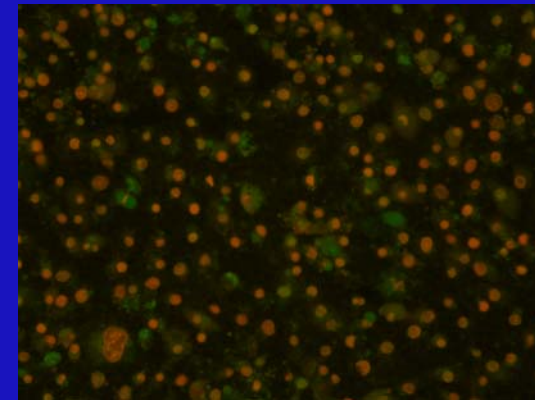
Control



NM 100 $\mu\text{g/ml}$

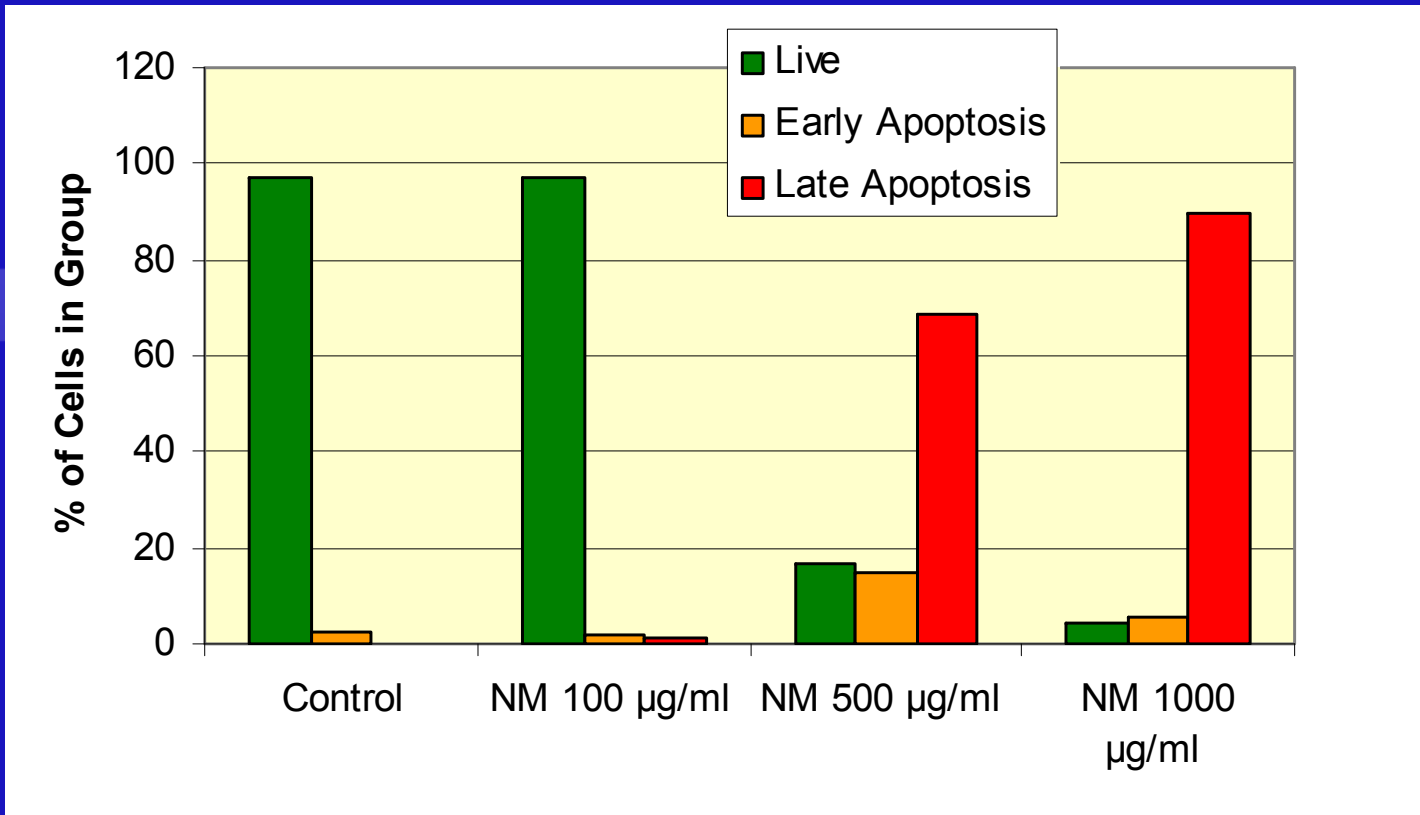


NM 500 $\mu\text{g/ml}$



NM 1000 $\mu\text{g/ml}$

Effect of NM on RCC 786-0 Apoptosis



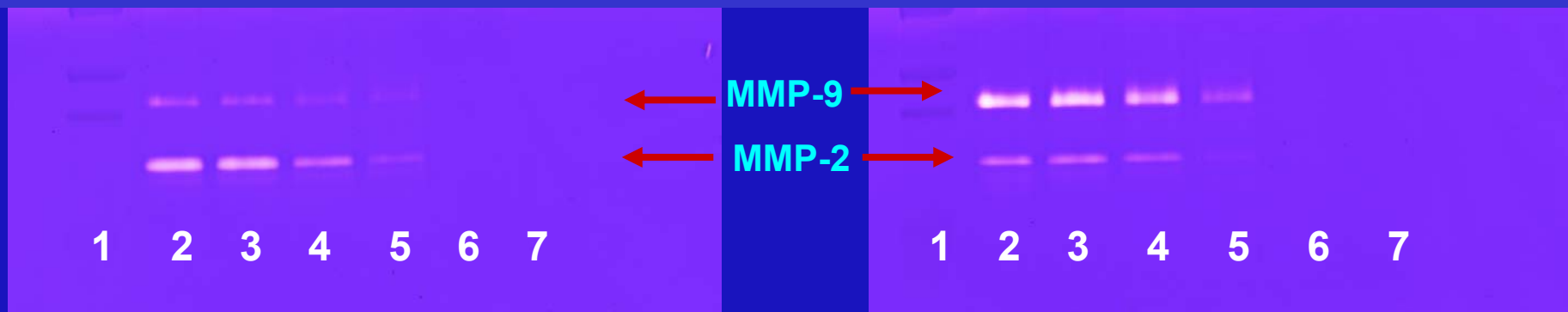
Effect of NM on RCC 786-0 MMP Secretion

Gelatinase Zymography

Zymography demonstrated secretion of MMP-2 by uninduced human RCC and both MMP-2 and -9 by PMA (200 ng/ml)-treated cells. NM inhibited secretion of both MMPs in a dose-dependent fashion with virtual total inhibition of MMP-2 at 500 $\mu\text{g/ml}$ concentration and MMP-9 at 100 $\mu\text{g/ml}$

Untreated RCC 786-0 cells

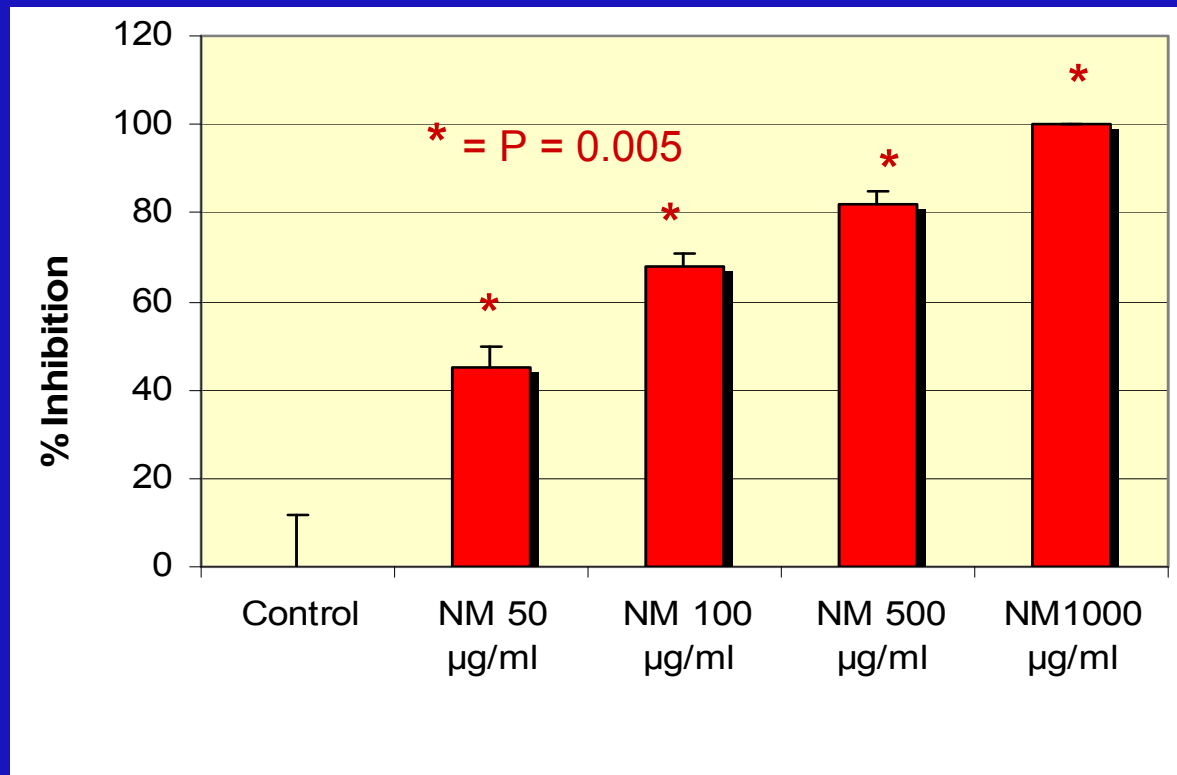
PMA (200 ng/ml-treated) RCC 786-0 cells



Legend: 1 - Markers, 2- Control, 3-7 NM 10, 50, 100, 500, 1000 $\mu\text{g/ml}$

Effect of NM on RCC 786-0 Invasion through Matrigel

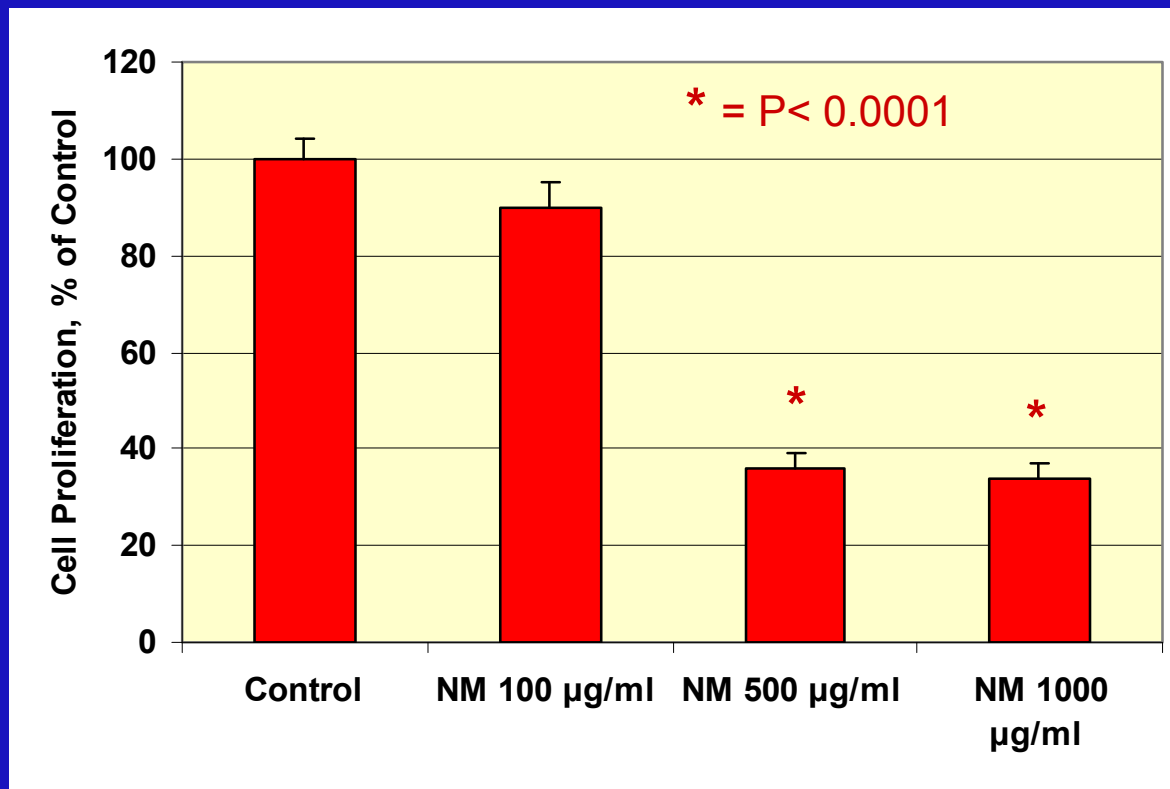
The invasion of human RCC 786-0 cells through Matrigel was significantly inhibited in a dose-dependent manner, reducing invasion by 82% at 500 $\mu\text{g/ml}$ and 100% at 1000 $\mu\text{g/ml}$ NM.



Effect of NM on Melanoma A2058 Toxicity

MTT Assay – 24h

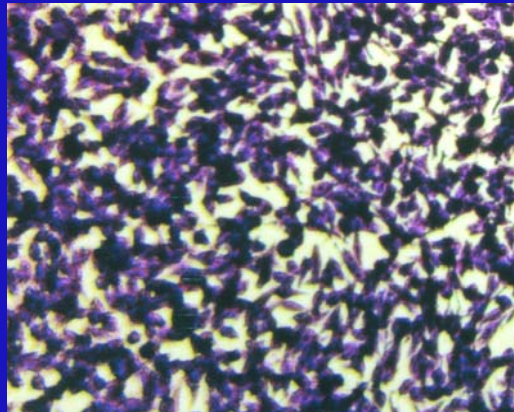
NM showed slight toxicity to melanoma A2058 cells at 100 $\mu\text{g/ml}$ NM, and 64% and 66% at 500 and 1000 $\mu\text{g/ml}$, respectively



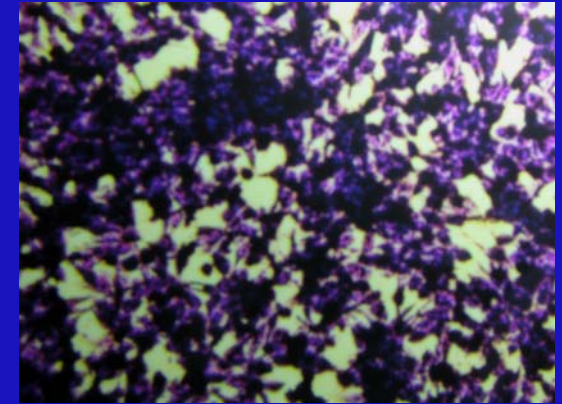
Effect of NM on Melanoma A2058 Morphology

H&E staining

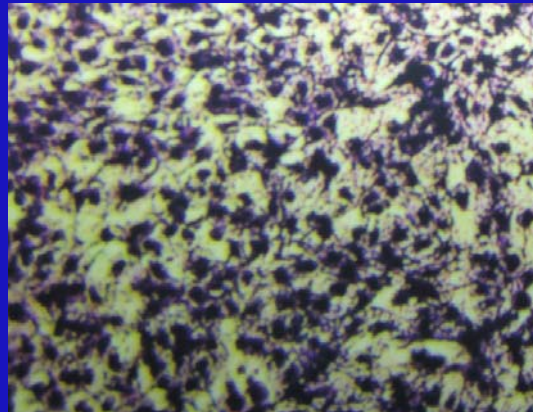
Melanoma A2058 cells treated with 100, 500 and 1000 $\mu\text{g/ml}$ NM showed obvious apoptotic cells by H&E staining. Apoptotic cells showed shrinkage with dark stained and condensed nuclei and strong acidophilic cytoplasm.



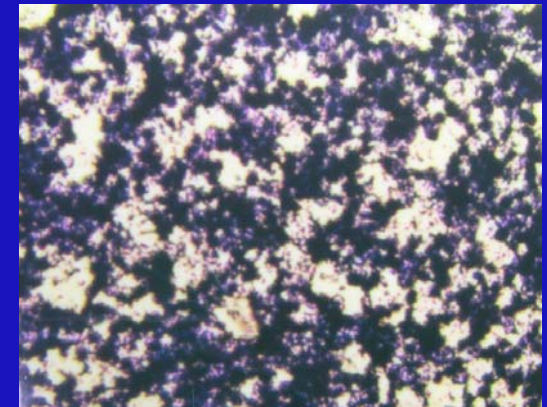
Control



NM 100 $\mu\text{g/ml}$



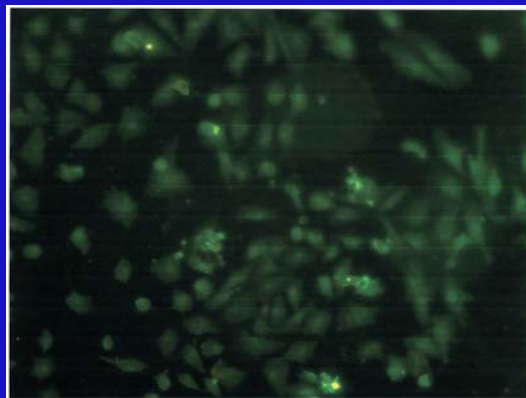
NM 500 $\mu\text{g/ml}$



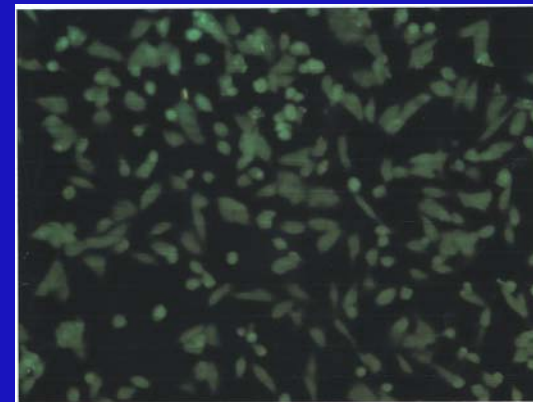
NM 1000 $\mu\text{g/ml}$

Effect of NM on Melanoma A2058 Apoptosis: Live green caspase detection kit

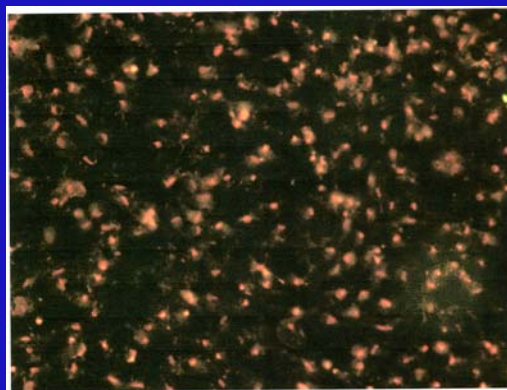
Melanoma A2058 cells exposed to various concentrations of NM demonstrated a considerable number of early and late apoptotic cells at 100 $\mu\text{g/ml}$ NM and a significantly increased number of apoptotic cells at 500 and 1000 $\mu\text{g/ml}$ NM.



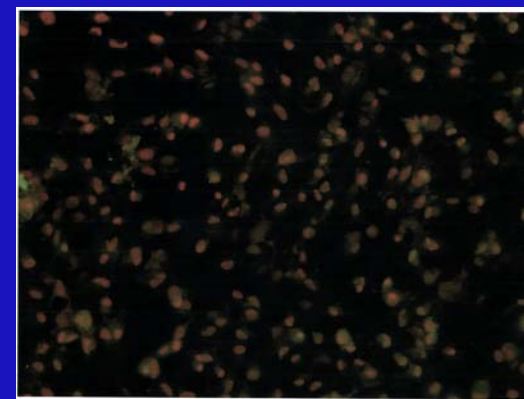
Control



NM 100 $\mu\text{g/ml}$

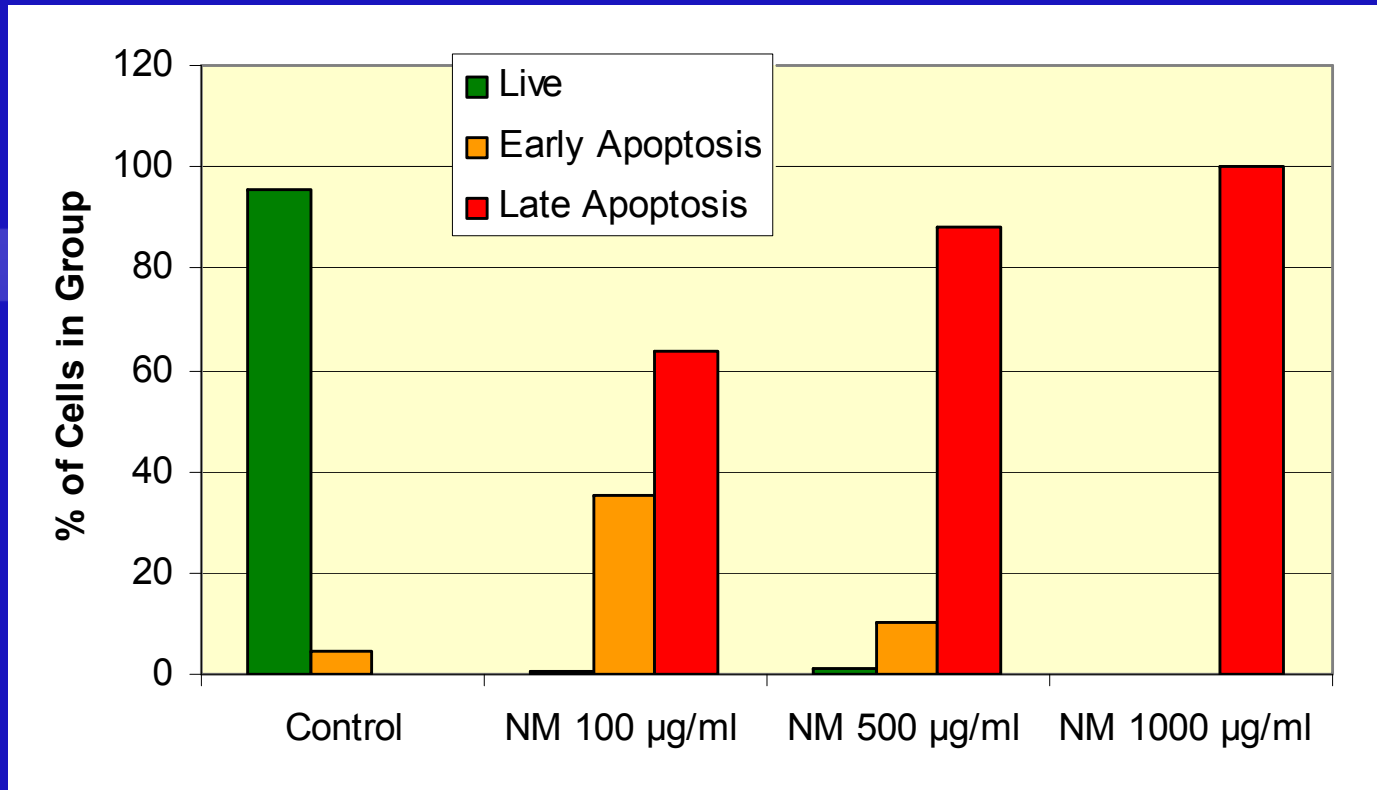


NM 500 $\mu\text{g/ml}$



NM 1000 $\mu\text{g/ml}$

Effect of NM on Melanoma A2058 Apoptosis



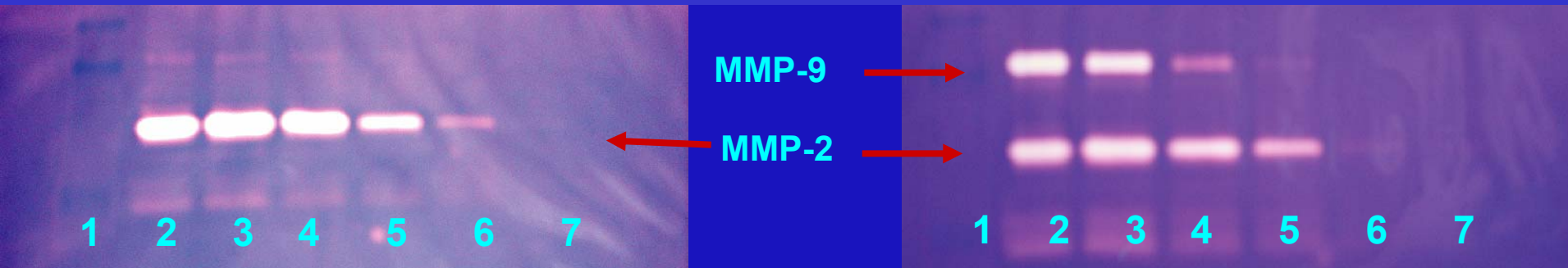
Effect of NM on Melanoma MMP Secretion

Gelatinase Zymography

Zymography detected MMP-2 secretion by human melanoma A2058 cells and induced MMP-9 secretion with PMA (200ng/ml) treatment. NM inhibited both MMP-2 and MMP-9 activity in a dose-dependent fashion with virtual complete blockage of MMP-9 at 100 $\mu\text{g/ml}$ and MMP-2 at 500 $\mu\text{g/ml}$.

Untreated melanoma A2058 cells

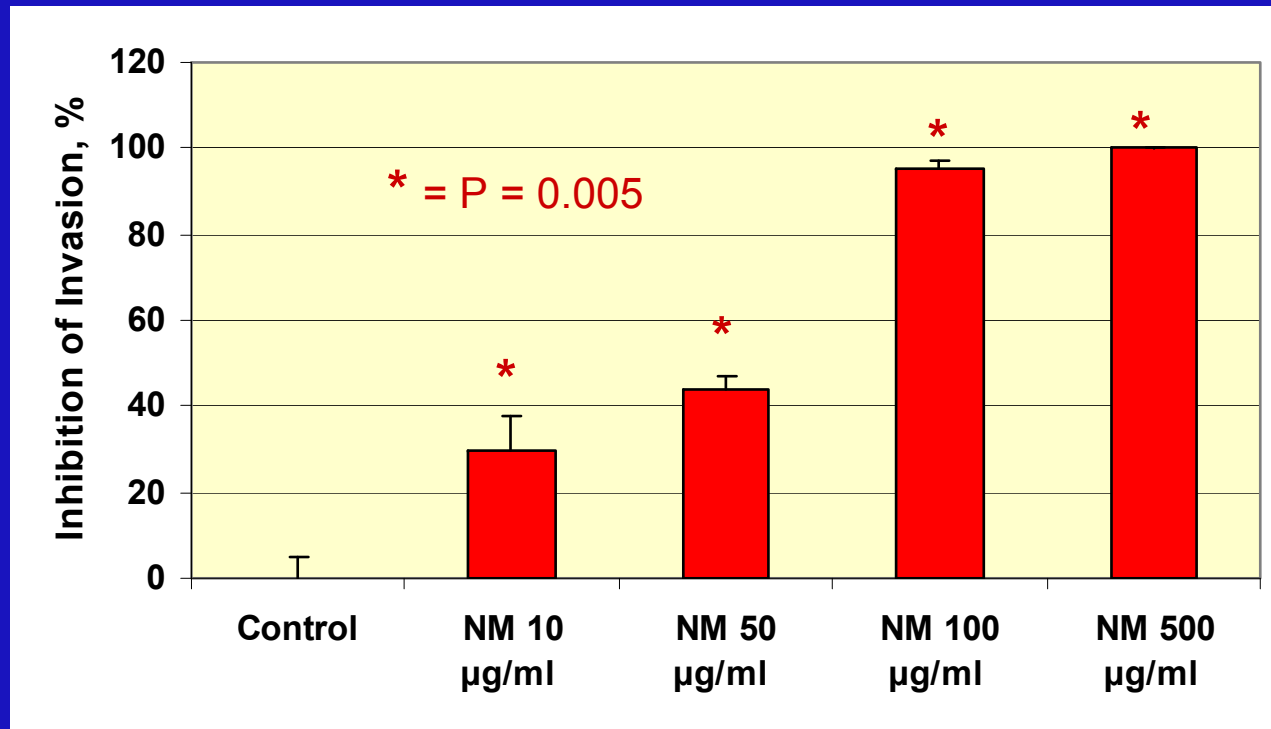
PMA (200 ng/ml-treated) A2058 cells



Legend: 1 - Markers, 2- Control, 3-7 NM 10, 50, 100, 500, 1000 $\mu\text{g/ml}$

Effect of NM on Melanoma Cell Invasion Through Matrigel

Invasion of melanoma A2058 cells through Matrigel was inhibited by 30%, 44%, 95% and 100% by 10, 50, 100, and 500 $\mu\text{g/ml}$ of NM, respectively.



Conclusions

Our results suggest that the nutrient mixture plays an important role in suppressing RCC and melanoma cell proliferation, inducing apoptosis, inhibiting MMP-2 and MMP-9 secretion, and inhibiting invasion through Matrigel, suggesting its therapeutic potential as an anticancer agent in managing patients with RCC and melanoma.

For further information on research activities, publications, and testimonials, visit our research website at:

[http:// www.drrathresearch.org](http://www.drrathresearch.org)