#2357 A Novel Nutrient Mixture Suppresses the Invasion Activity of Human Uterine Leiomyosarcoma Cell Line SK-UT-1 by Inhibiting MMPs

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1. Introduction:

Uterine leiomyosarcoma (LMS), a rare malignant tumor with poor prognosis, arises from the smooth muscle lining the uterine wall. The exact causes of LMS are not known, but genetic and environmental risk factors are associated with it. LMS continue to be a deadly disease. Most patients receive multimodality therapy including surgery followed by chemotherapy or radiation therapy. Current chemotherapeutic drugs are minimally effective with progression of disease in 80% of treated patients. Adjuvant therapy after optimal cytoresection does not decrease the rate of recurrence. Aggressive surgical cytoreduction at the time of initial diagnosis offers the possibility of prolonged survival.

2. Objective:

We investigated the effect of a nutrient mixture (NM) on proliferation, invasive potential, MMPs, and apoptosis of human LMS cell line SK-UT-1. NM is a specific mixture of lysine, proline, ascorbic acid and green tea extract that was shown to have potent antitumor activity through inhibiton of MMPs.

3. Methods:

Human LMS cell line SK-UT-1 (ATCC) was grown in DEME media with 10% FBS and antibiotic in 24-well tissue culture plates. At near confluence, the cells were tested with NM at 0, 50, 100, 250, 500 and 1000 µg/ml in triplicate at each dose. Cell proliferation was assayed by MTT assay, MMPs by gelatinase zymography, invasion through Matrigel, apoptosis using live green caspase detection kit (Molecular Probe), and morphology by H&E staining.

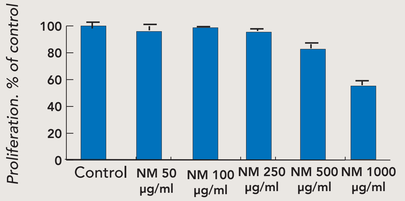
Composition of the Nutrient Mixture (NM)

Nutrient	Proportion
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

4.Results:

1. NM was not toxic to SK-UT-1 cells at 250 µg/ml, but exhibited 20% and 40% toxicity at 500 and 1000 µg/ml (Fig 1).

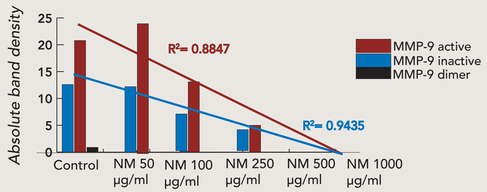
Figure 1 - Effect of NM on SK-UT-1 cell proliferation



2. Zymography did not show bands for either MMP-2 or MMP-9 in normal SK-UT-1 cells. However, PMA treatment stimulated MMP-9 expression, both inactive and active forms in equal proportions. NM inhibited the secretion of both active and inactive forms of MMP-9 in a dose response fashion. Faint bands were observed at 500 µg/ml with total inhibition at 1000 µg/ml NM (Fig 2A-B).

Figure 2A- Effect of NM on MMP-9 secretion by PMA- treated SK-UT-1 cells

Figure 2B - Densitometry of MMP-9 secretion by PMA treated SK-UT-1 cells



3. Invasion of SK-UT-1 cells through Matrigel was inhibited by 9%, 35%, 94% and 100% at 50, 100, 250 and 500 µg/ml, NM respectively (Fig 3A-B).

Figure 3A- Effect of NM on inhibition of invasion by SK-UT-1 cells

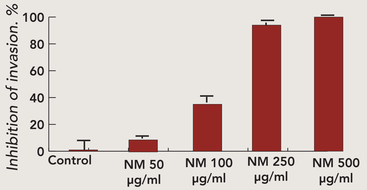
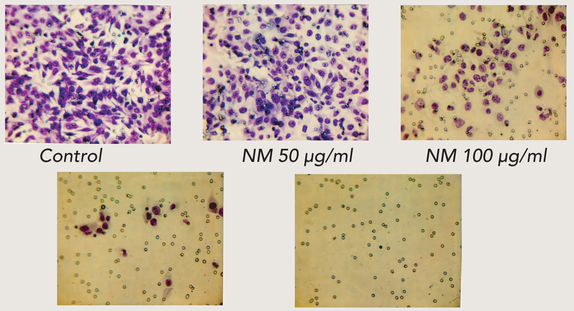


Figure 3B- Invasion photomicrographs



4. NM induced slight apoptosis at 100 µg/ml and significant at 250 and 500 µg/ml (Fig 4A-B).

Figure 4 - Apoptosis quantitative analysis

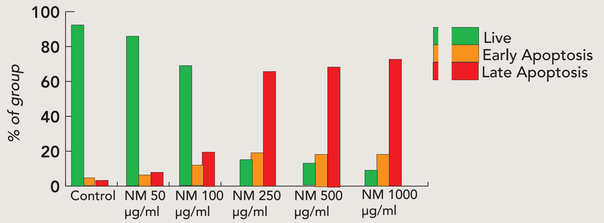
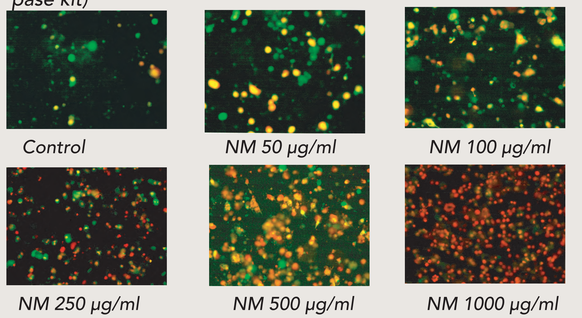
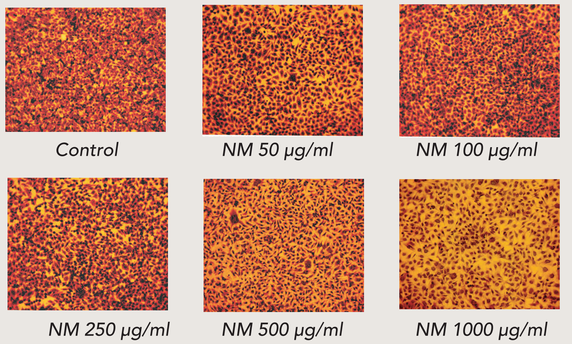


Figure 4B - Apoptosis photomicrographs (live green caspase kit)



5. H& E showed slight changes at the highest dose (Fig 5).

Figure 5 - Morphology H&E staining



5. Conclusions:

Our results indicate that NM significantly inhibited MMP secretion, invasion through Matrigel, and apoptosis, important parameters for cancer prevention, suggesting NM has the potential for therapeutic use in the treatment of LMS.