Marked Inhibition of Tumor Growth, MMP Secretion and Invasion by a Nutrient Mixture on Head and Neck Squamous Carcinoma Cell LIne FaDu: in Vitro and in Vivo Studies

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Background:

Head and neck squamous cell carcinomas (HNSCC), the sixth most common malignancy in the United States, are known for their aggressive growth and propensity to invade and metastasize. We investigated the effect of a novel nutrient mxiture (NM) containing ascorbic acid, lysine, proline, and green tea extract on human HNSCC cell line FaDu in vivo, in athymic nude mice bearing HNSCC FaDu xenografts, and in vitro, evaluating viability, MMP secretion, invasion and morphology.

Methods:

In Vivo

1. After one week of isolation, 5-6 week old athymic male nude mice were inoculated with 3 x 10^6 FaDu cells subcutaneoulsy and randomly divided into two groups; group A was fed a regular diest and group B a regular diet supplemented with 0.5% NM.
2. Four weeks later, the mice were sacrificed and their tumors were excised, weighted and processed for histology.

In Vitro

1. In vitro, FaDu cells were cultured in appropriate media and exposed to NM at 0, 10, 50, 100, 500 and 1000 µg/ml concentration in triplicate. Cells were also treated with PMA (200 ng/ml) for MMP-9 induction.
2. Cell proliferation was assessed by MTT assay.
3. MMP secretion was measured by gelatinase zymography.
4. Invasion was evaluated through Matrigel.
5. Cell morphology was evaluated by H&E staining.

Composition of Nutrient Mixture (NM)

Nutrient	Per Stock Solution
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 strongly inhibited the growth of tumors by 55% (p =0.0002), as shown in Figure 1. Histologically, tumor specimens from both groups consisted of a large central necrotic region and a peripheral collar of viable tumor tissue consistent with a poorly differentiated carcinoma. Necrosis involved 60-70% of the control group specimen and 40-50% of the supplemented group specimen (Figure 2)

Figure 1 - Effect of NM on growth of HNSCC FaDu xenografts in male nude mice

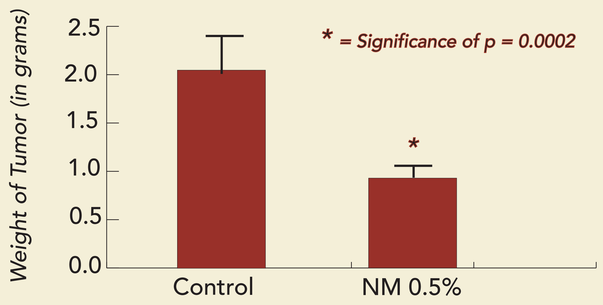
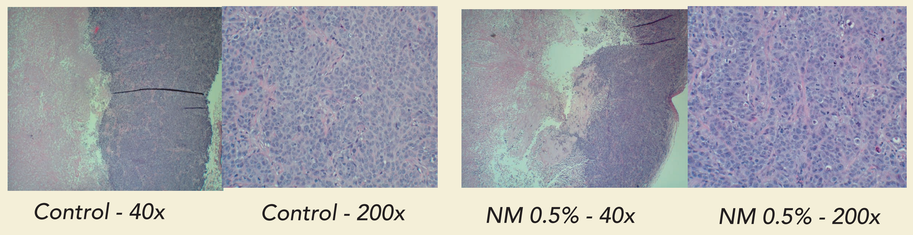


Figure 2 - Histopathology of tumor xenografts of HNSCC FaDu in nude mice

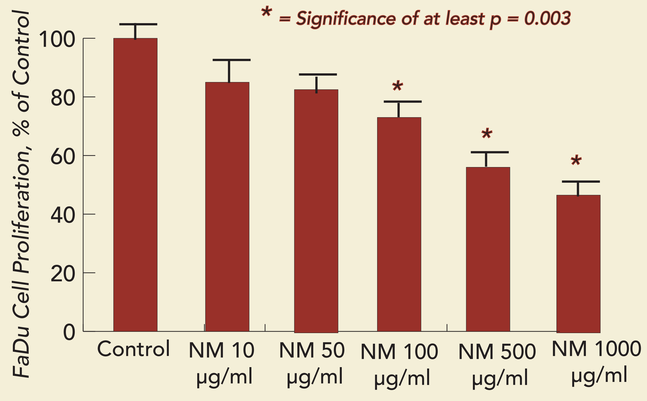


Control Group- peripheral collar of viable neoplastic tissue and central area of necrosis.

Supplemented Group (NM 0.5%)- peripheral collar of viable neoplastic tissue and central area of necrosis.

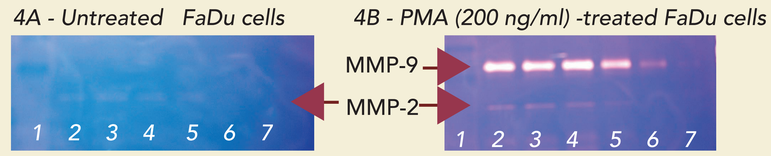
2. NM exhibited dose response toxicity on HNSCC FaDu cells in vitro, with maximum toxicity of 53% (p=0.0003) over the control at 1000 µg/ml, as shown in Figure 3.

Figure 3 - Effect of NM on proliferation/toxicity of head and neck squamous carcinoma cell line FaDu



3. Zymography showed a faint band representing MMP-2 and a strong band representing PMA-induced MMP-9 secretion. NM inhibited secretion of both MMPs in a dose-dependent manner, with virtual total inhibition at 1000 µg/ml, as shown in Figure 4.

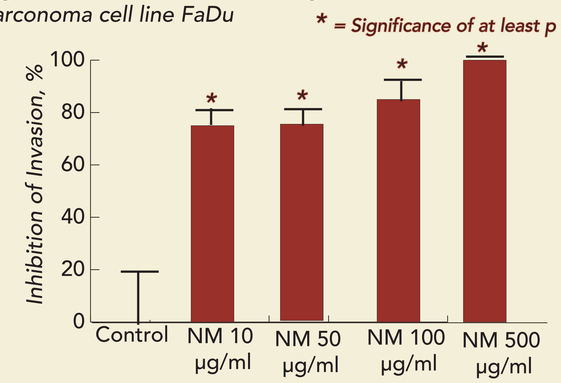
Figure 4 - Effect of NM on MMP secretion by head and neck squamous carcinoma cell line FaDu



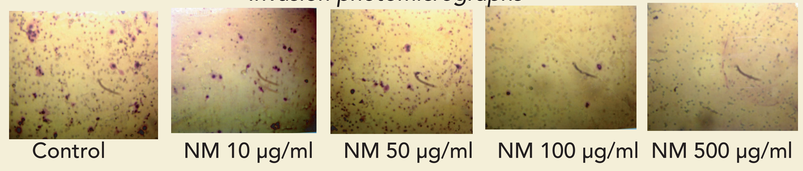
Legend: 1 - Markers, 2 - Control, 3-7 NM 10, 50, 100, 500, 1000 µg/ml

4. NM significantly inihbited FaDu invasion through Matrigel by 75%, 85%, and 100% at 50, 100, and 500 µg/ml , as shown in Figure 5.

Figure 5 - Effect of NM on Matrigel invasion of head and neck squamous carconoma cell line FaDu

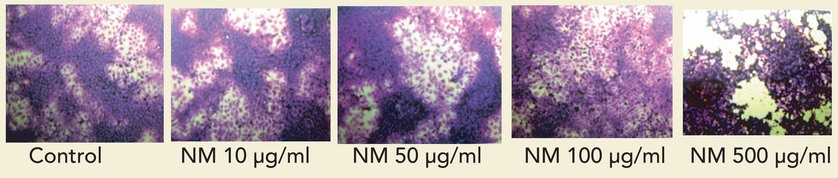


Invasion photomicrographs



5. H&E staining did not indicate any changes in HNSCC FaDu cell morphology up to 100 µg/ml NM, as shown in Figure 6.

Figure 6 - Effect of NM on morphology of FaDu cells: H&E



Conclusion

NM has a great potential for therapeutic use in the treatment of HNSCC by significantly suppressing tumor growth and significantly inhibiting MMP secretion and invasion of HNSCC cells in vitro.