Inhibition of MMP Secretion, Invasion and Growth of Tongue Cancer Cell Line SC-255 by a Nutrient Mixture

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Introduction

Oral cancer represents about 3% of all cancers. The American Cancer Society estimates that approximately 30,000 new cases of oral cancer are diagnosed annually in the U.S.A. with about 20,000 occurring in men and 10,000 in women. Although the exact cause of tongue cancer remains unknown, it is associated with tobacco product use. Treatment of tongue cancer is based on the stage of the disease and involves radiation, chemotherapy and surgery. Nutrient supplementation has been reported to support recovery from cancer and maintenance of good health.

Objective

We investigated the effect of a novel nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract on human tongue cancer cell line SC-255 in vitro, evaluating viability, MMP secretion, invasion and morphology.

Methods and Materials

Tongue cancer cell line SC-255 (ATCC) was grown in modified Dulbecco's Eagle medium with 10% fetal bovine serum and antibiotics and treated with NM at 0, 10, 50, 100, 500, and 1,000 μg/ml in triplicate at each dose. Cells were also treated with PMA (200 ng/ml) for MMP-9 induction. Cell proliferation was assayed by MTT, MMPs by zymography, invasion through Matrigel, and morphology by H&E staining.

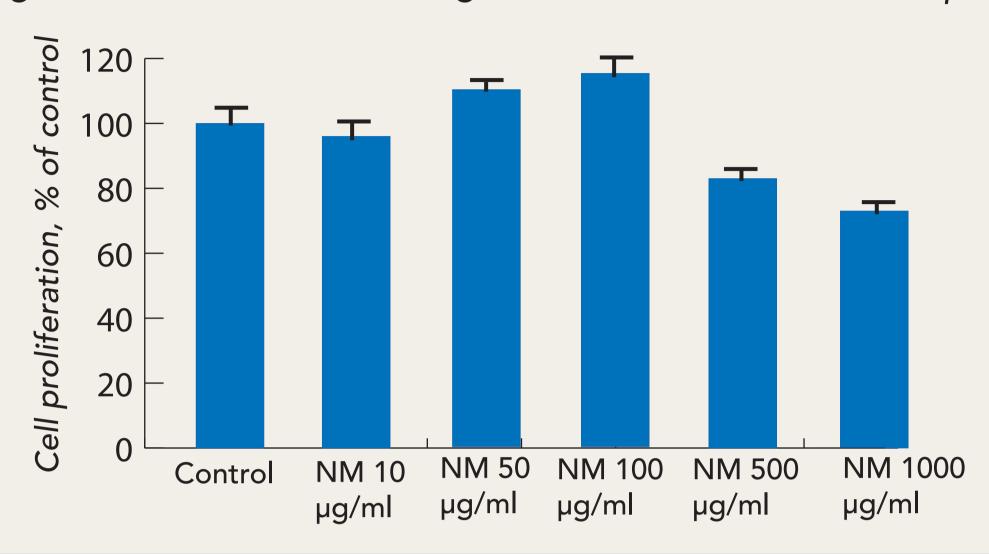
Composition of the Nutrient Mixture (NM)

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 (8	30%
polyphenol)	1000 mg
Selenium	30 µg
Copper	2 mg
Manganese	1 mg

Results

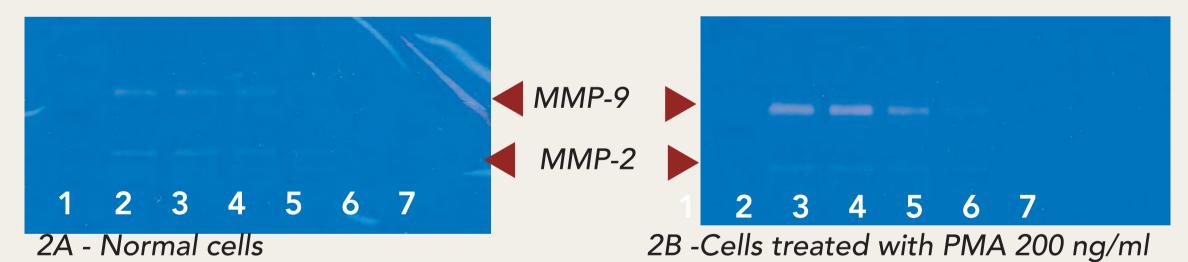
1. NM was not toxic to SC-255 tongue cancer cells at 100 μg/ml, but exhibited 20% and 30% toxicity at 500 and 1000 µg/ml, as shown in Figure 1.

Figure 1 - Effect of NM on tongue carcinoma cell line SC-255 proliferation



2. Zymography demonstrated two bands corresponding to MMP-2 and MMP-9 secretion by tongue carcinoma cell line SC-255. PMA treatment enhanced MMP-9 secretion. NM inhibited secretion of both MMP-2 and MMP-9 in a dose-dependent fashion, with total virtual inhibition at 500 µg/ml, as shown in Figures 2A and 2B. Densitometry analyses are shown in Figures 2C-2F.

Figure 2 - Effect of NM on MMP-2 and MMP-9 secretion by tongue carcinoma cell line SC-255



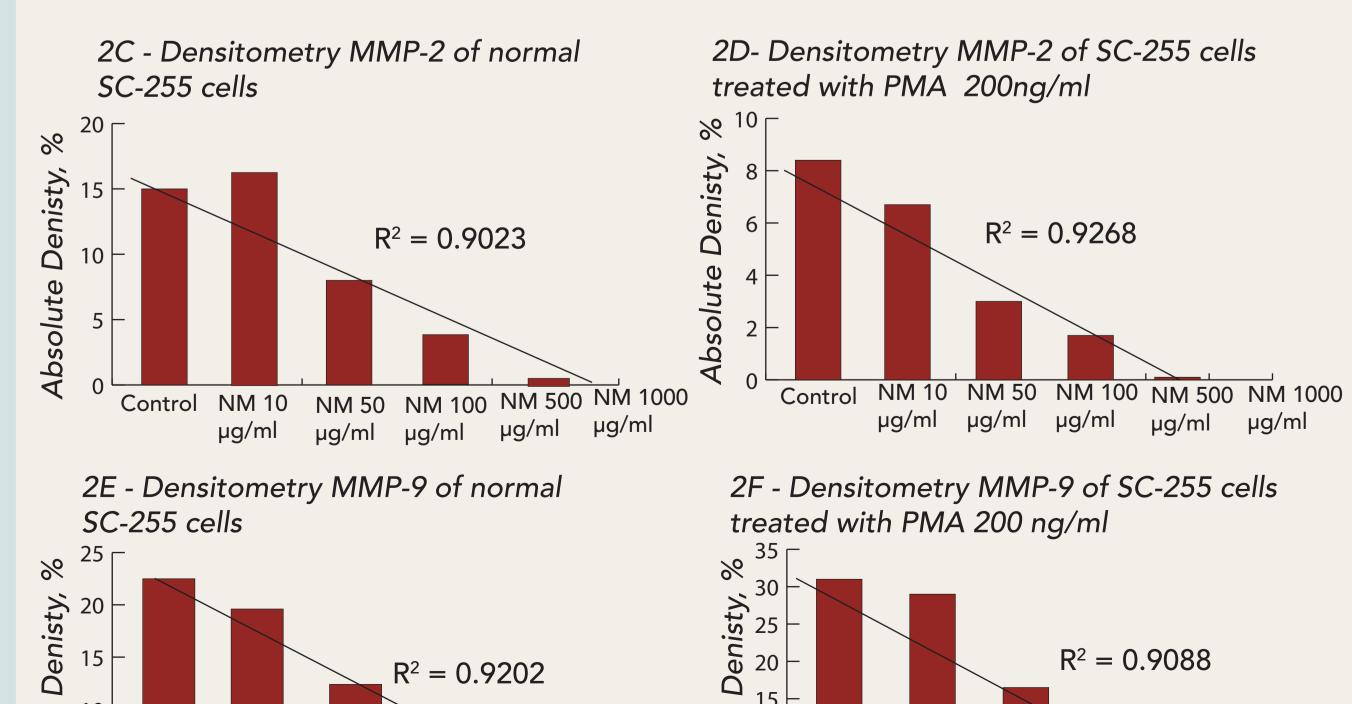
Legend - 1 Markers, 2 Control, 3-7 NM 10, 50, 100, 500, 1000 µg/ml

 $R^2 = 0.9202$

NM 10 NM 50 NM 100 NM 500 NM 1000

μg/ml μg/ml μg/ml μg/ml

Absolute



3. Invasion through Matrigel was inhibited by 40%, 80% and 100% at 100, 500 and 1000 μ g/ml NM respectively.

Figure 3 - Effect of NM on tongue carcinoma cell line SC-255 invasion through Matrigel

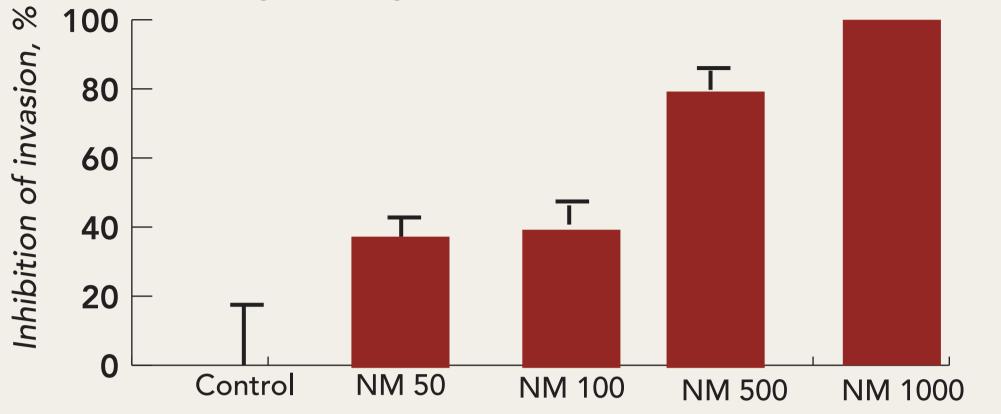
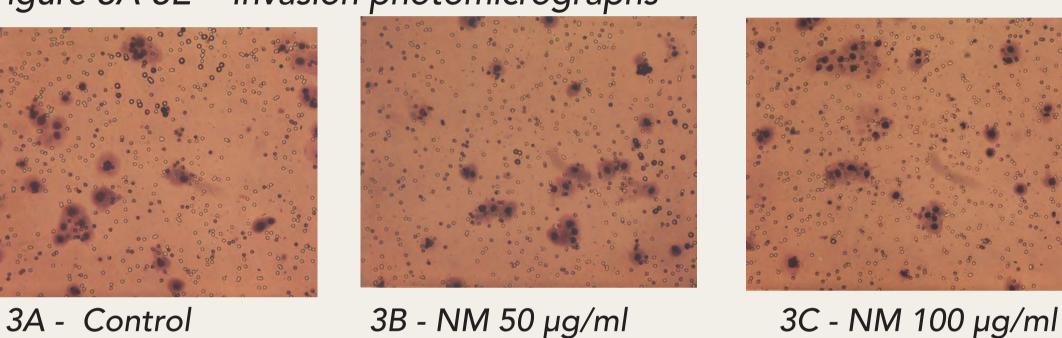


Figure 3A-3E - Invasion photomicrographs







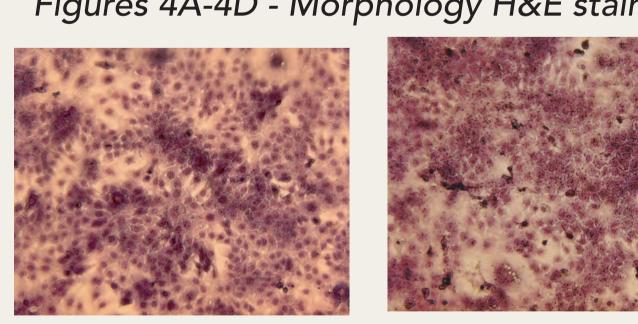
3D - NM 500 μg/ml

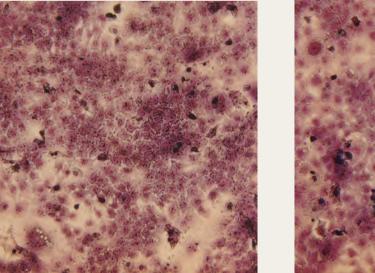
3E - NM 1000 μg/ml

4. H&E staining of tongue carcinoma SC-255 cells exposed to NM showed slight changes only at the highest concentration of NM, as shown in Figures 4A-4D.

4B - NM 50 μg/ml

Figures 4A-4D - Morphology H&E stains





4C - NM 100 μg/ml

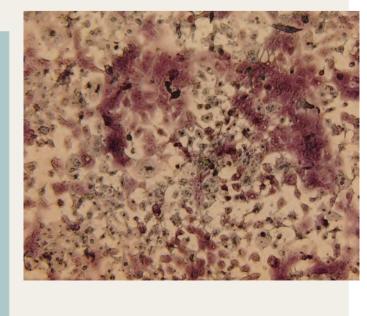
Conclusions:

4A - Control

NM 10 NM 50 NM 100 NM 500 NM 1000

μg/ml μg/ml μg/ml

NM significantly inhibited growth, MMP secretion, and invasion through Matrigel, important parameters for cancer prevention, suggesting NM has potential for therapeutic use in treatment of tongue cancer.



4D - NM 500 μg/ml