In Vitro and in Vivo Inhibition of Human Fanconi Anemia Head and Neck Squamous Carcinoma by a Novel Nutrient Mixture

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1. Objective:

Fanconi Anemia (FA), an autosomal recessive disease associated with chromosomal instability, is characterized by progressive bone marrow failure, birth defects and high propensity to develop malignancies. Head and neck squamous cell carcinoma (HNSCC) and acute myeloid leukemia are the major causes of mortality and morbidity in FA patients. The objective of this study was to investigate the antineoplastic activity of NM, a novel antineoplastic nutrient mixture (containing lysine, proline, ascorbic acid and green tea extract) that demonstrates a broad spectrum of antitumor activity against a number of human cancer cell lines.

2. Methods and Materials:

A. Human FAHNSCC cell line OHSU-974 (Fanconi Anemia Research Fund, Oregon Health & Science University, Portland, Oregon, USA) was cultured in RPMI medium supplemented with 20% FBS and antibiotics. At near confluence, cells were treated in triplicate with different concentrations of NM: 0, 50, 100, 250, 500 and 1000 µg/ml. Cells were also treated with PMA (100 ng/mL) to induce MMP-9 activity. Cell proliferation was detected by MTT assay, secretion of MMPs by gelatinase zymography, invasion through Matrigel, migration by scratch test and morphology by H&E staining.

B. In vivo, athymic male nude mice (n=12) were inoculated with $3x10^6$ OHSU-974 cells subcutaneously and randomly divided into two groups: group A was fed a regular diet and group B a regular diet supplemented with 1% NM. Four weeks later, the mice were sacrificed and their tumors were excised, weighed and processed for histology.

Compostion 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

3. Results:

3.1 NM inhibited the growth of head and neck squamous carcinoma cell line of Fanconi Anemia patient OHSU-974 tumor by 48% (p=0.0009) and tumor burden by 50% (p=0.0003). See Figures 1A, 1B.

Figure 1A - Effect of NM on tumor weight of OHSU-974 xenograft

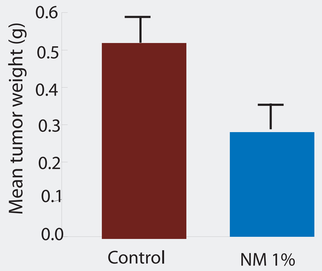
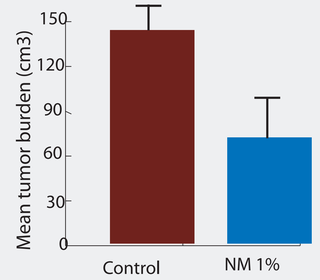
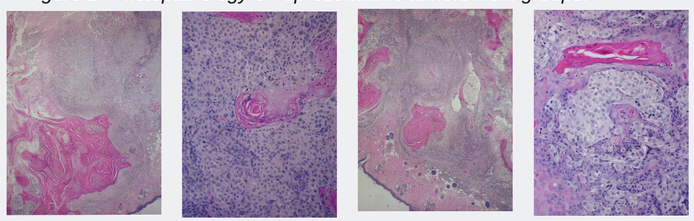


Figure 1B - Effect of NM on tumor burden of OHSU-974 xenograft



3.2 The tumors in both groups were shown to be irregularly round, ulcerated, skin, subcutaeous masses consistent with squamous cell carcinoma, as shown in Figure 2. The NM group tumors were smaller than the control tumors.

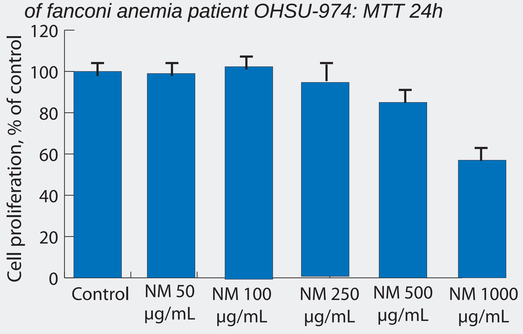
Figure 2 - Histopathology of representative tumors from groups.



2A-Control 40x 2B-Control 200x 2C-NM 1% 40x 2D-NM 1% 200x

3.3 NM demonstrated no effect on proliferation, but at 1000 µg/mL showed 40% toxicity (Figure 3).

Figure 3 - Effect of NM on head & neck squamous carcinoma cell line of fanconi anemia patient OHSU-974: MTT 24h

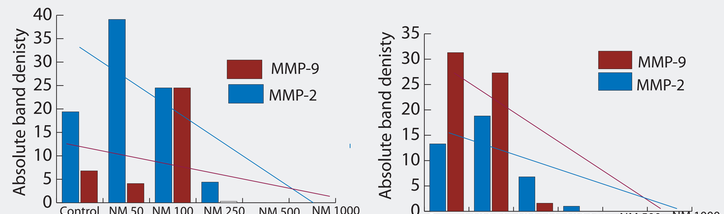


3.4 Zymography revealed MMP-2 and PMA-induced MMP-9 secretion. NM suppressed OHSU-974 cell secretion of both MMPS in a dose-dependent manner, with virtual inhbition at 500 µg/ml (Figure 4).

Figure 4- Zymograms of untreated (4A) and PMA-treated (4B) cells

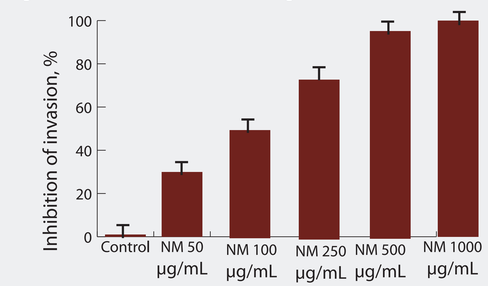
Legend: 1 - Markers, 2- Control, 3-7 - NM 50, 100, 250, 500, 1000 µg/ml

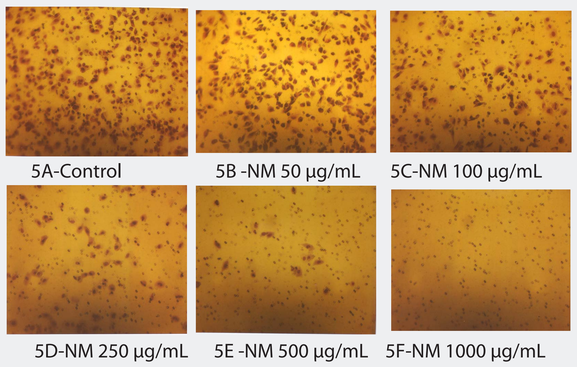
Figure 4- Densitometric analysis of untreated (4C) and PMA-treated (4D) cells



3.5 NM inhibited OHSU-974 invasion through Matrigel in a dose-dependent fashion with total block at 1000 µg/ml (Figure 5). Photomicrographs shown in 5A-5F.

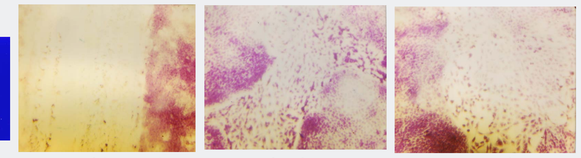
Figure 5 - Effect of NM on Matrigel invasion of OHSU-974 cells



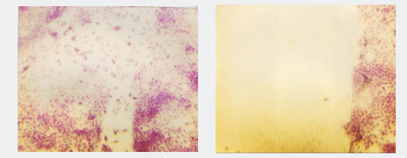


3.6 NM inhibited OHSU-974 migration (scratch test) in a dose-dependent fashion with total block at 250 µg/ml (Figure 6).

Figure 6 - Effect of NM on OHSU-974 cell migration



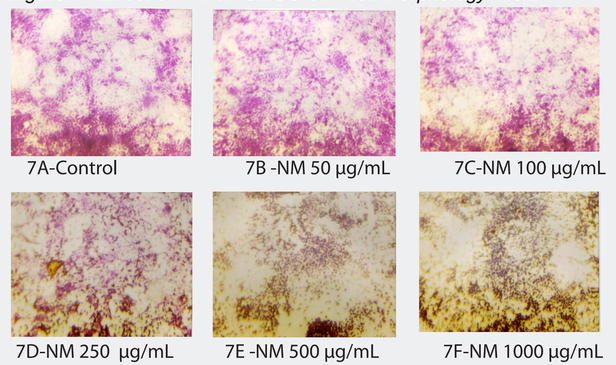
6A-Control (0 time) 6B -Control 24h 6C-NM 50 µg/mL 24h



6D-NM 100 µg/mL 24h 6E -NM 250 µg/mL 24h

3.7 H&E staining showed no morphological changes below 500 µg/mL (Figures 7A-F).

Figure 7 - Effect of NM on OHSU-974 cell morphology: H&E



7A-Control 7B -NM 50 µg/mL 7C-NM 100 µg/mL
7D-NM 250 µg/mL 7E -NM 500 µg/mL 7F-NM 1000 µg/mL

4. Conclusion:

The results suggest that NM has potential therapeutic use in the treatment of human FA HNSCC.

5. Translational Applicability:

Current treatment methods for Fanconi Anemia-associated cancers are generally ineffective. Thus, there is a need for development of effective therapeutic agents for these cancers with minimal toxicity. Our studies demonstrated that NM significantly inhibited the growth and tumor burden of FA HNSCC cell line OHSU-974 in vivo. In addition, invasive parameters, such as MMP-2 and -9 secretion and invasion were significantly inhibited by NM in vitro. These findings suggest the potential of NM in FA HNSCC treatment.