In Vitro Inhibition of Matrix Metalloproteinases, Invasion and Growth of Human FANCA and FANCC Lymphoblasts by a Unique Nutrient Mixture

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M. W. Roomi, V. Ivanov, A. Niedzwiecki, M. Rath
Dr. Rath Research Institute, Oncology, Santa Clara, CA

Introduction:

Fanconi anemia (FA) is a rare genetic disorder characterized by progressive anemia, birth defects, chromosome fragility and high propensity to development of cancer. Aplastic anemia and head and neck squamous cell carcinomas are the major causes of mortality and morbidity in FA patients. Matrix metalloproteinases (MMPs) have received much attention in recent years for their role in various malignancies, and have been implicated in tumor invasion and metastasis. Biological agents that prevent extracellular matrix (ECM) degradation by MMPs have been shown to be promising therapeutic approaches to cancer. A nutrient mixture (NM) containing ascorbic acid, lysine, proline and green tea extract showed significant anticancer activity against a number of cancer cell lines.

Objective:

We investigated the effect of NM on human FANCA and FANCC lymphoblasts for viability, MMP secretion and invasion.

Methods:

1. Human FANCA lymphoblasts GM13022 and FANCC lymphoblasts HCS536 were cultured in RPMI supplemented with 15% FBS and antibiotics.

2. The cells were then challenged with NM at 0, 10, 50, 100, 500, and 1000 µg/ml concentration in triplicate at each concentration.

3. Cell proliferation was assessed by counting cells stained with Trypan blue, invasion was evaluated through Matrigel and MMP activity by gelatinase zymography. Cells were also treated with PMA to induce MMP-9 activity.

4. **Composition of Nutrient Mixture (NM)**

Nutrient	Per Stock Solutions
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. The nutrient mixture (NM) exhibited 20% inhibition of HCS536 lymphoblast growth compared to the control at 10 µg/ml, and 40% at 50-1000 µg/ml concentration (Figure 1). However, NM was not toxic to GM13022 lymphoblasts, even at the highest concentration (Figure 2).

Figure 1 - Effect of NM on HCS536 Lymphoblast Growth

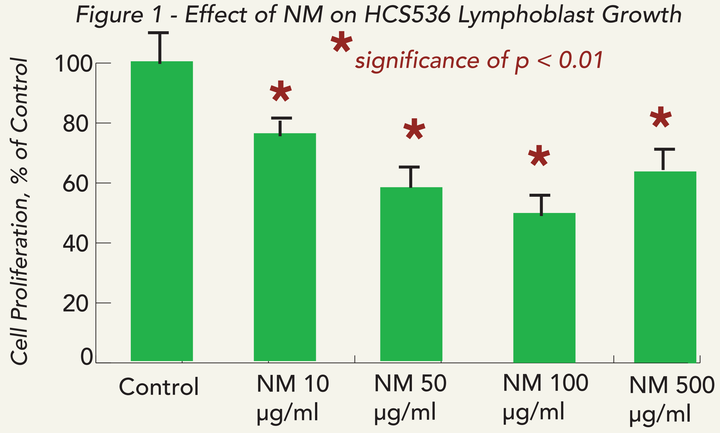
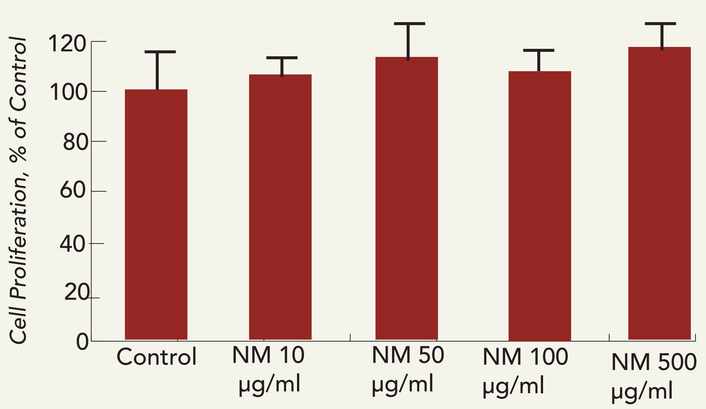
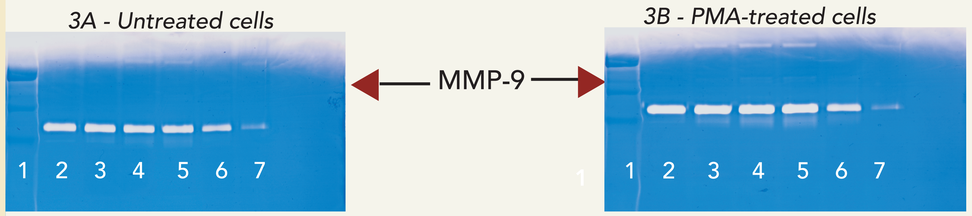


Figure 2 - Effect of NM on GM13022 Lymphoblast Growth



2. GM13022 lymphoblasts exhibited only MMP-9 secretion, which was enhanced by PMA (Figure 3). NM inhibited MMP-9 secretion at 500 µg/ml less than at 1000 µg/ml concentration. HCS536 lymphoblasts did not demonstrate MMP activity even with PMA stimulation.

Figure 3 - Effect of NM on GM13022 Lymphoblast MMP Secretion



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

3. Invasion through Matrigel was inhibited in HCS536 at 100 and 500 µg/ml by 27% and 93% (Figure 4). In GM13022, NM had little effect at 50 and 100 µg/ml, but at 500 µg/ml NM completely blocked invasion (Figure 5).

Figure 4 - Effect of NM on HCS536 Lymphoblast Matrigel Invasion

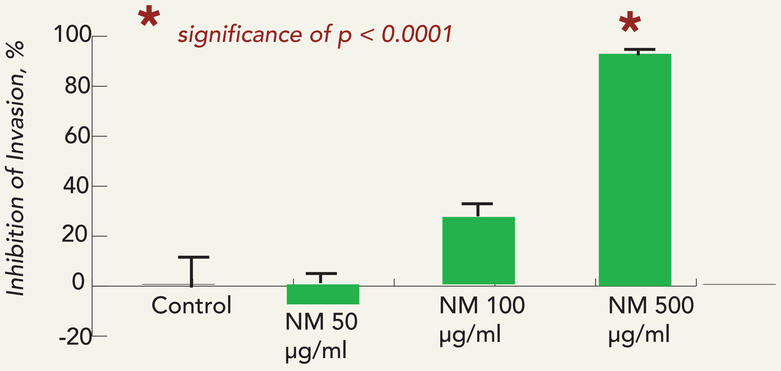
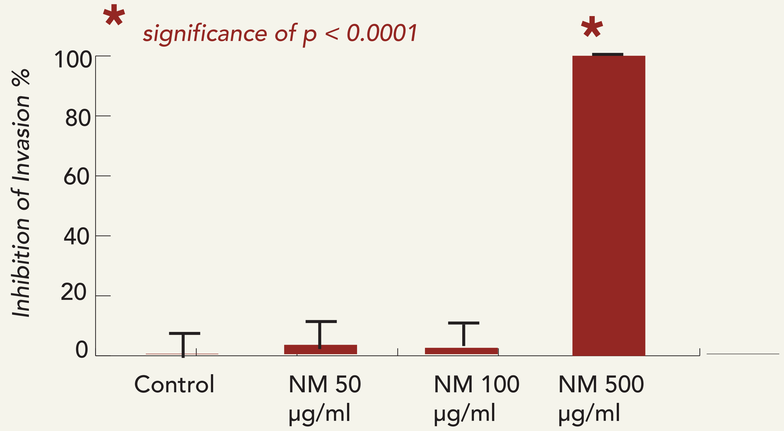


Figure 5 - Effect of NM on GM13022 Lymphoblast Matrigel Invasion



Conclusion:

The nutrient mixture inhibited MMP secretion and Matrigel invasion in FANCA, and invasion and proliferation in FANCC lymphoblasts, suggesting NM has a potential therapeutic use in the treatment strategy in FA neoplasia.