#4963 *In vivo* and *in vitro* effect of a nutrient mixture on murine 4T1 breast cancer cells

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Introduction

Breast cancer patients often have detectable or occult metastases at diagnosis and most patients will develop metastatic lesions during the course of the disease.

Objective

We investigated the effect of a nutrient mixture (NM) containing ascorbic acid, lysine, proline, and green tea extract on murine breast cancer 4T1, a unique metastatic breast cancer model that has the capacity to metastasize efficiently to sites affected in human breast cancer.

Materials and Methods

1. After one week of isolation, 5-6 week old female Balb/C mice were inoculated with $5x10^5$ 4T1 cells into the mammary pad and randomly divided into two groups; group A was fed a regular diet and group B a regular diet supplemented with 0.5% NM.
2. After four weeks, the mice were sacrificed and their tumors, lungs, livers, kidneys, hearts and spleens were excised and processed for histology.
3. Dimensions (length and width) of tumors were measured using a digital caliper, and the tumor burden was calculated using the following formula: 0.5 x length x width.
4. We also tested the effect of NM *in vitro* on 4T1 cells, measuring cell proliferation by MTT assay, MMP secretion by zymography, invasion through Matrigel , migration by scratch test and morphology by H&E staining.

Composition of Nutrient Mixture (NM)

Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbate)	700 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 inhibited tumor weight and burden of 4T1 tumors by 50% (p=0.02) and 53.4% (p<0.0001), respectively, as shown in Figure 1.

Figure 1A - Tumor weight

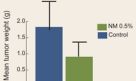


Figure 1B - Tumor burden

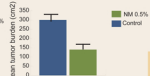
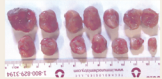
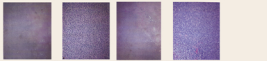


Figure 1C - Gross tumors from groups



2. Histologically, both groups demonstrated irregularly round subcutaneous tumors with large central areas of tumor necrosis involving 70% of the tumor mass in the control mice and 50-70% in the supplemented mice. See Figure 2

Figure 2- Tumor histopathology



3. Lung metastasis was profoundly inhibited by NM supplementation: mean number of colonies was reduced by 87% (p<0.0001) and mean weight of lungs by 60% (p=0.0001) compared to control mice. See Figure 3.

Figure 3A- Mean number of colonies

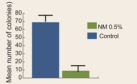
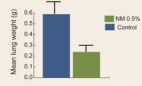
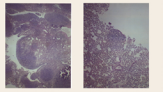


Figure 3B- Mean lung weight



4. Histopathology confirms the inhibition of lung metastasis in NM supplemenated mice, as shown in Figure 4.

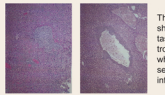
Figure 4- Histopathology of lungs



Multiple metastases were observed in the lungs of control mice in contrast to few, small metastatic lesions in lungs of NM supplemented mice. Neoplastic cells were large, irregularly round, with prominent large, irregularly round nuclei and scant cytoplasm

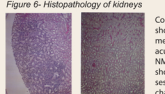
4. Metastases to liver, spleen, kidney and heart were significantly reduced with NM supplementation, as seen in Figures 5-8. No significant differences were found between control and NM supplemented mean organ weights.

Figure 5- Histopathology of livers



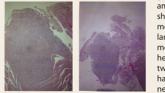
The control liver showed focal metastasis and severe neutrophilic infiltration, while the NM had severe perivascular infiltration

Figure 6- Histopathology of kidneys



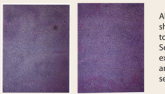
Control kidneys showed subscapular, metastatic lesions and acute infarction, while NM kidney sections showed no metastases or specific changes.

Figure 7- Histopathology of hearts



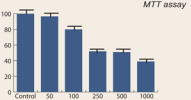
Among the control group heart sections examined, four of five showed myocardial metastatic lesions, one large and three smaller metastases. In the NM heart sections examined, two of four sections each had a metastatic lesion near the base of the heart.

Figure 8- Histopathology of livers



All control group spleen sections showed severe, extramedullary hematopoiesis and 2-3 small metastases. Sections of NM spleen showed severe, extramedullary hematopoietic activity and a small, metastatic lesion in one section

Figure 9- Effect of NM on 4T1 cell proliferation: MTT assay



5. In vitro, NM exhibited dose dependent inhibition of cell proliferation (p<0.0001), with 50% toxicity over the control at 250 and 500 µg/ml concentrations, and 61% at 1000 µg/ml, as shown in Figure 9.

6. Zymography demonstrated MMP-2 and MMP-9 secretion by 4T1 cells which was inhibited by NM in a dose dependent fashion, with virtual total inhibition of both at 1000 µg/ml.

Figure 10- Effect of NM on 4T1 cell MMP-2 and-9 secretion

10A - Untreated 4T1 cells *10B - PMA (100 ng/ml)-treated 4T1 cells*

7. Invasion of 4T1 cells through Matrigel and migration through scratch test were inhibited by NM in a dose dependent manner, with total block of invasion at 250 µg/ml and of migration at 1000 µg/ml, as shown in Figures 11 and 12, respectively.

Figure 11- Effect of NM on 4T1 invasion through Matrigel

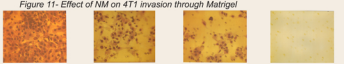


Figure 12 - Effect of NM on 4T1 cell migration:scratch test

Conclusions

These results suggest that NM has therapeutic potential in treatment of breast cancer.