#1127 Downregulation of urokinase plasminogen activator and matrix metalloproteinases and upregulation of their inhibitors by a novel nutrient mixture in human prostate cancer cell lines PC-3 and DU-145

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1. Introduction:

Prostate cancer is the most frequently diagnosed cancer among men in the U.S. Cancer turns deadly when it invades and metastasizes to other parts of the body. Proteases play a key role in tumor cell invasion and metastasis due to their ability to digest basement membrane and ECM components. Two families of proteases, serine (u-PA) and MMPs-2 and-9 are necessary for invasive and metastatic potential. U-PA and MMPs are expressed in many tumor cell lines. Strong clinical and experimental evidence shows that elevated levels of u-PA and MMPs are associated with tumor growth, cancer progression, metastasis and shortened survival in patients. MMP activities are regulated by specific tissue inhibitors of metallproteinases (TIMPs).

2. Objective:

Biological agents that prevent ECM degradation have been shown to be a promising therapeutic approach to cancer. A nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract showed anticancer activity against a number of cancer cell lines. We investigated the effect of NM on the activity of u-PA, MMPs and their inhibitor TIMPs on human prostate cancer cell lines PC-3 and DU-145.

3. Materials and Methods:

Human prostate cancer cell lines PC-3 and DU-145 (ATCC) were grown in MEM media with 10% FBS and antibiotics in 24-well tissue culture plates. At near confluence, the cells were treated with NM at 0, 50, 100, 250, 500 and 1000 µg/ml in triplicate at each concentration. U-PA activity was carried out by fibrin zymography on 10% SDS-PAGE gels containing fibrinogen and plasminogen, MMPs by gelatinase zymography and TIMPs were analyzed by reverse zymography.

Composition of the Nutrient Mixture (NM)

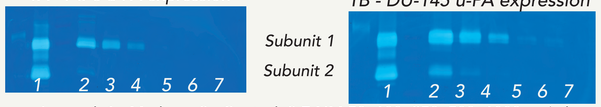
Nutrient	Proportion
Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbate)	710 mg
L-Lysine	1000 mg
L-Proline	750 mg
L-Arginine	500 mg
N-Acetyl Cysteine	200 mg
Standardized Green Tea Extract (80% polyphenol)	1000 mg
Selenium	30 µg
Copper	2 mg
Manganese	1 mg

4. Results:

1. U-PA activity was detected in both PC-3 (Fig 1A and 1C) and DU-145 (Fig 1B and 1D) prostate cancer cell lines, showing two bands corresponding to molecular weights 35 and 33 kD. NM inhibited their expression in a dose response manner, with significant reduction in u-PA activity at 250 µg/ml NM.

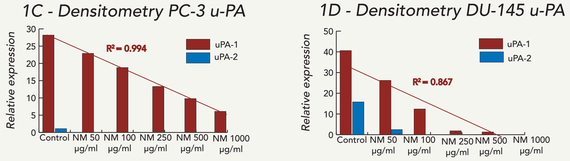
Figure 1 - Prostate cancer cell line PC-3 and DU-145 u-PA expression

1A - PC-3 u-PA expression *1B - DU-145 u-PA expression*



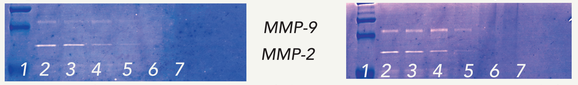
Legend: 1 - Markers, 2 - Control, 3-7 NM 50, 100, 250, 500, 1000 µg/ml

1C - Densitometry PC-3 u-PA *1D - Densitometry DU-145 u-PA*



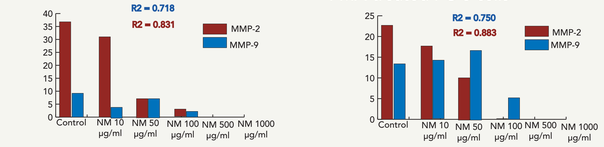
2. PC-3 on gelatinase zymography showed bands for MMP-2 and MMP-9 (Fig 2A-D). NM inhibited the expression in a dose-dependent manner. The activity of MMP-2 and MMP-9 was significantly inhibited at 250 µg/ml NM with total inhibition at 500 µg/ml. Interestingly DU-145 did not exhibit MMP bands.

Figure 2 - Prostate cancer cell line PC-3 MMP-2 and -9 expression



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

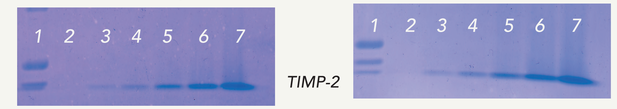
2C - Densitometry MMP expression normal PC-3 cells *2D - Densitometry MMP expression PMA-treated PC-3 cells*



3. Activity of TIMPs was upregulated in both cancer cell lines PC-3 (Fig 3A and 3C) and DU-145 (Fig 3B and 3D) in a dose-dependent manner. Minimum activity was expressed at 50 µg/ml and maximum at 1000 µg/ml.

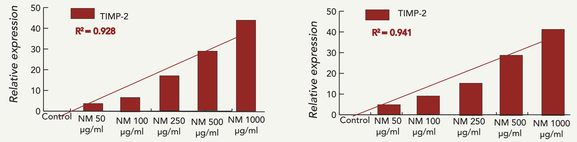
Figure 3 - Prostate cancer cell line PC-3 and DU-145 TIMPs expression

3A - PC-3 TIMPs expression *3B - DU-145 TIMPs expression*



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

3C - Densitometry PC-3 TIMP-2 *3D - Densitometry DU-145 TIMP-2*



4. Analysis revealed a positive correlation between PC-3 u-PA and MMP expression (Fig 4A) and a negative correlation between expression of u-PA and TIMP-2 (Fig 4B) and MMPs and TIMP-2 (Fig 4C). A negative correlation was found between DU-145 expression of TIMP-2 and u-PA (Fig 4D).

Figure 4A - Correlation between PC-3 u-PA and MMP expression

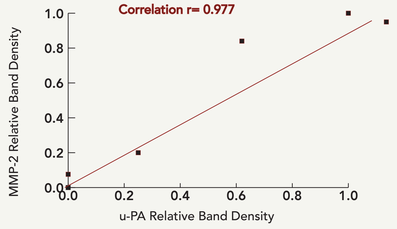


Figure 4B - Correlation between PC-3 u-PA and TIMP-2 expression

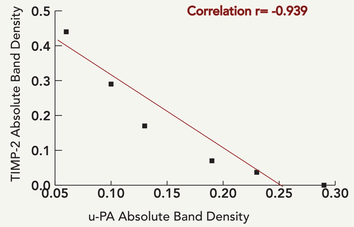


Figure 4C - Correlation between PC-3 MMPs and TIMP-2 expression

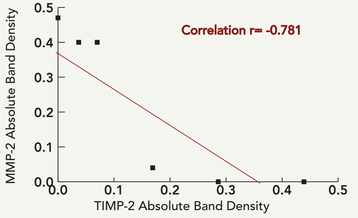
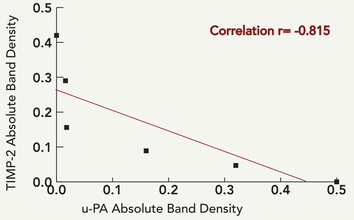


Figure 4D- Correlation between DU-145 u-PA and TIMP-2 expression



5. Conclusion:

Based on these studies it is tempting to suggest that NM could potentially be developed as a new anticancer agent that inhibits u-PA and MMPs and increases TIMPs.