Effect of a novel nutrient mixture on Fanconi Anemia fibroblast and normal human dermal fibroblast: a comparison

M.W. Roomi, N.W. Roomi, A. Niedzwiecki and M. Rath
Dr. Rath Research Institute, Santa Clara, CA, USA

27th Annual Fanconi Anemia Scientific Symposium
September 17-20, 2005 Toronto, Canada

Objective:

Fanconi anemia (FA) is a fatal heterogeneous autosomal recessive disorder, characterized by progressive bone marrow failure, congenital defect and cancer predisposition. Cell culture from FA fibroblast (FAF) displays certain abnormalities as compared to normal human dermal fibroblast (NHDF). This prompted us to investigate the effect of a novel nutrient mixture (NM) containing ascorbic acid, lysine, proline and green tea extract, which has demonstrated a broad spectrum of pharmacological activities, on FAF compared to NHDF.

Materials and Methods:

1. We investigated the *in vitro* effect of NM on FAF and NHDF cell proliferation by MTT assay, MMPs secretion by zymography, morphology by H&E staining and apoptosis by green caspase assay.
2. FAF (FA-A:PD20, FA-A:PD220) and NHDF were cultured in modified Dulbecco Eagle media. At near confluence, the cells were treated with different concentrations of NM (0, 10, 50, 100, 500 and 1000 µg/ml) in triplicate. The cells were also treated with PMA to induce MMP-9 activity.

Composition of the Nutrient Mixture (NM)

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

Conclusions:

Our data demonstrate that NM exhibited different responses toward FAF and NHDF. This may in part be due to elevated chromosomal break, deletion and hypersensitivity to cross linking agents, a DNA repair disorder in FAF that is lacking in NHDF.

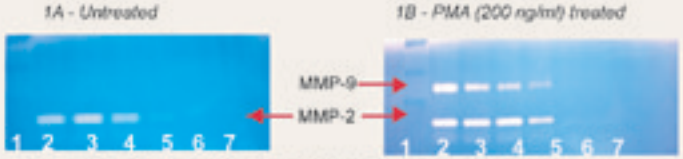
Translational Applicability:

FA patients have a 20% chance of developing cancers. FA fibroblasts differ from normal fibroblasts due to an impaired DNA repair process with chromosomal break, deletion and hypersensitivity to cross linking agents. Thus, we studied the difference in FA and normal fibroblasts response to NM treatment. The results are significant since they showed MMP-9 secretion by FAF, not by NHDF, a parameter of cancer cell degradation of the extracellular matrix and mediator of cancer cell growth, invasion/metastasis and angiogenesis. NM significantly inhibited FAF MMP-9 secretion.

Results:

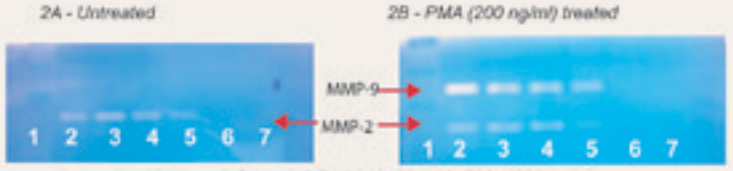
1. Zymography demonstrated MMP-2 and MMP-9 on PMA stimulation in FAF; NM inhibited the activity of both MMP-2 and MMP-9 in a dose response fashion with total block at 500 µg/ml. In contrast, NHDF exhibited only MMP-2. NM inhibited MMP-2 activity in a dose-dependent manner with total block at 1000 µg/ml (Figures 1 -3).

Figure 1 - Effect of NM on MMP secretion by human FA fibroblasts FA-A:PD20



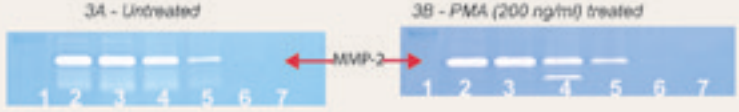
Legend: 1-Markers, 2-Control, 3-7 NM 10, 50, 100, 500, 1000 µg/ml

Figure 2 - Effect of NM on MMP secretion by human FA fibroblasts FA-A:PD220



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

Figure 3- Effect of NM on MMP secretion by human dermal fibroblasts NHDF



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

2. NM had no effect on FAF cell viability in both cell lines compared to control. In contrast NM exhibited 20% toxicity at 50 and 100 µg/ml and 33% at 500 and1000 µg/ml in NHDF cells (Figure 4A-C).

Figure 4A - Effect of NM on growth of human FA fibroblasts FA-A:PD20 at 24 hrs (MTT

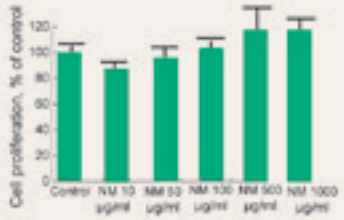


Figure 4B - Effect of NM on growth of human FA fibroblasts FA-A:PD220 at 24

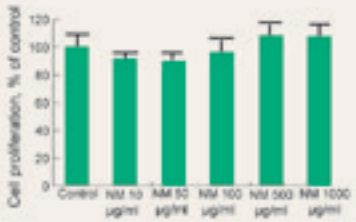
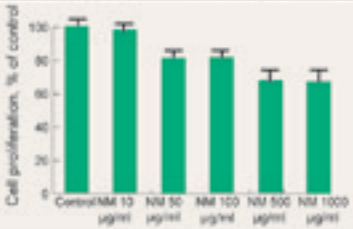


Figure 4C - Effect of NM on growth of human dermal fibroblasts at 24h: MTT assay



3. H&E staining did not indicate any morphological changes in FAF nor induced apoptosis at higher NM concentrations, as seen by caspases assay. Although no morphological changes in NHDF were noted up to NM 100 µg/ml, progressive changes in cell shrinkage, rounding and nuclear condensation, pertaining to apoptosis, were observed at higher concentrations. These changes were consistent with the results from the green caspases apoptosis assay. See Figures 5 and 6.

Figure 5A - Effect of NM on morphology of human FA fibroblasts FA-A:PD20: H&E staining

Figure 5B - Effect of NM on morphology of human FA fibroblasts FA-A:PD220: H&E staining

Figure 5C - Effect of NM on morphology of human dermal fibroblasts: H&E staining

Figure 6A - Effect of NM on apoptosis of human FA fibroblasts FA-A:PD220: caspase

Figure 6B- Effect of NM on apoptosis of human dermal fibroblasts: caspase