IN VITRO INHIBITION OF MATRIX METALLOPROTEINASES, INVASION AND GROWTH OF FANCONI ANEMIA HUMAN FANCA AND FANCC LYMPHOBLASTS BY NUTRIENT MIXTURE

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Aim: Fanconi anemia is a rare genetic disorder with high propensity for development of cancers, such as aplastic anemia, leukemia and head and neck cancers. Collagen digesting matrix metalloproteinase (MMP) enzymes have been implicated in for their role in various malignancies and to promote metastasis. Biological agents that prevent extracellular matrix digestion by the MMPs have been shown to be promising therapeutic approaches to cancer. In this study, we investigated effects of a nutrient mixture (NM) containing, ascorbic acid, lysine, proline and green tea extract, on human FA and FANCC lymphoblasts for viability, MMP secretion and invasion. Methods: Human FA lymphoblasts GM13022 and HCS536 were challenged with NM at concentration range within 10–1000 µg/ml. Cell toxicity was assessed by Trypan blue dye exclusion test. Invasion was evaluated through Matrigel and gelatinase zymography for MMP activity. Results: NM was toxic in dose dependent mode to HCS536 cells but not to GM13022 cells. GM13022 cells but not HCS536 cells exhibited MMP-9 secretion, which was inhibited by NM. Matrigel invasion was inhibited in HCS536 cells at 100 and 500 µg/ml by 27% and 93%, respectively. In GM13022 cells, the NM showed completely blocked Matrigel invasion at 500 µg/ml. Conclusion: NM inhibited MMP secretion and Matrigel invasion in FANCA and inhibited invasion and induced toxicity in FANCC lymphoblasts. These results suggest that the NM may have therapeutic potential in Fanconi anemia associated neoplasia. Key Words: Fanconi anemia, nutrient mixture, MMP, Matrigel invasion.
Gelatinase zymography was performed as described [6]. In brief, the culture media (20 µl) were mixed with the sample buffer and loaded for SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE) with trisglycine SDS buffer. Samples were not boiled before electrophoresis. Following electrophoresis, the gels were washed twice in 2.5% Triton X-100 for 30 min at room temperature to remove SDS. The gels were then incubated at 37 °C overnight in CaCl2 at pH 8.0 and stained with 0.5% Coomassie Blue R250 in 50% methanol and 10% glacial acetic acid for 30 min and de-stained producing clear bands against an intensely stained background. Gelatinase zymograms were scanned using a CanoScan 9950F Canon scanner at 1.200 dpi. The intensity of the bands was evaluated using a pixel-based densitometer program Un-Scan-It, Version 5.1, 32-bit (Silk Scientific Corporation), at a resolution of 1 Scanner Unit (1/100 dpi), and expressed as a percentage of control.

The results were expressed as mean ± SD. Data was analyzed by independent t-test. NM exhibited dose-dependent death of HCS536 lymphoblast within the range of 10–500 µg/ml (Fig. 1, a) but not GM13022 even at the highest concentration (Fig. 1, b).

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**Fig. 1.** Effect of NM on FA lymphoblasts: a — NM was toxic to HCS536 lymphoblast growth (p < 0.01); b — NM was not toxic to GM13022 lymphoblast growth.

GM13022 lymphoblasts exhibited only MMP-9 secretion (Fig. 2, a), which was enhanced by PMA (100 ng/ml) as shown in Fig. 2, b. NM inhibited MMP-9 secretion 50% at 500 µg/ml, and 90% at 1000 µg/ml concentration (Fig. 2, c). In contrast, HCS536 lymphoblasts did not demonstrate MMP activity, even with PMA stimulation (figure not shown).

**Fig. 2.** Effect of NM on GM13022 lymphoblasts on MMP secretion: a — untreated cells; b — PMA (100 ng/ml) treated cells (Legend: 1 — marker, 2 — control, 3–7 — NM 10, 50, 100, 500, 1000 µg/ml); c — densitometry analysis of the effect of NM on untreated cells shown in a (R² = 0.8296)

Invasion through Matrigel was inhibited in HCS536 at 100 µg/ml and 500 µg/ml by 27% and 93% as shown in Fig. 3. In GM13022 cells, the NM had little effect at 50 µg/ml and 100 µg/ml, but at 500 µg/ml NM completely blocked the invasion.

**Fig. 3.** Effect of NM on Matrigel Invasion: a — Matrigel invasion of HCS536 lymphoblasts (p < 0.0001); b — GM13022 lymphoblasts
Our studies demonstrate that NM was toxic to HCS536 cells, inhibited secretion of MMP-9 in GM13022 and invasion in both FA lymphoblasts. 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 ECM degradation by MMPs has shown to be a promising preventative approach to cancer.

FANCA mutations are the most prevalent, accounting for approximately two-thirds of all FA cases, FANCC and FANCG account for 25% of cases of FA mutations [7]. In addition to predisposition to many types of bone marrow disorders, some of these mutations are thought to be linked at a molecular level to many other DNA damage-repair disorders [8]. The genetic defects associated with FA patients are also found to be closely related to those of breast cancer with BRCA2 mutation [9, 10].

Head and neck squamous cell carcinoma and acute myeloid leukemia are the most frequently occurring cancers in FA patients. Current treatment methods available for FA associated cancers are generally ineffective and particularly toxic. Thus, there is a need for development of effective therapeutic agents for these cancers with minimal toxicity. In a recent study, we demonstrated that the NM inhibited MMP expression, invasion, induced apoptosis in immortalized FA patient fibroblasts — FA-A:PD20, FA-A:PD220 cell lines [11]. In another study, we have shown that the NM also significantly inhibited growth and tumor burden of OHS-947 FA-HNSCC cell line in vivo and inhibited proliferation, secretion of MMP and Matrigel invasion in vitro [12]. Moreover, in contrast to the toxic effects of current chemotherapeutic approaches, the NM has been shown to be safe in other studies [13].

The present study further supports these benefits of the NM in FA cells and therefore indicates that the NM is a safe and effective approach to support FA patients.

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REFERENCES