A Nutrient Mixture Modulates Ovarian ES-2 Cancer Progression By Inhibiting Xenograft Tumor Growth And Cellular MMP Secretion, Migration And Invasion (#2023)

SOT
55th Annual Meeting
March 13-17, 2016

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1.Objective

Epithelial ovarian carcinoma, which occurs mainly in post-menopausal women, is the leading cause of death from gynecological malignancy and the fifth most common cancer in the U.S. Since ovarian cancer often remains clinically silent, the majority of patients with ovarian carcinoma have advanced intraperitoneal metastatic disease at diagnosis, resulting in a poor prognosis. Long-term survival of patients with ovarian cancer remains poor, due to metastasis and recurrence. We investigated the effect of a nutrient mixture (EPQ) containing ascorbic acid, lysine, proline, quercetin and green tea extract *in vivo* and *in vitro* on human ovarian cancer ES-2 cell line.

2.Materials and Methods

In vivo

1. Athymic female nude mice (n=12) were inoculated with $3x10^6$ ES-2 cells subcutaneously and randomly divided into two groups: group A was fed a regular diet and group B a regular diet supplemented with 0.5% EPQ.
2. Four weeks later, the mice were sacrificed and their tumors were excised, weighed and processed for histology. 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.

In vitro

We tested the effect of EPQ *in vitro* on ES-2 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 EPQ

Nutrients	Per stock solution
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
Quercetin	50 mg

3.Results

1. EPQ inhibited tumor weight and burden of ES-2 tumors by 59.2% ($p < 0.0001$) and 59.7% ($p<0.0001$), respectively (Figure 1).

Figure 1A - Effect of EPQ on tumor weight

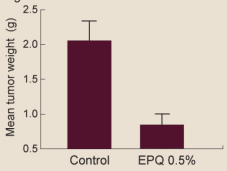


Figure 1B - Effect of EPQ on tumor burden

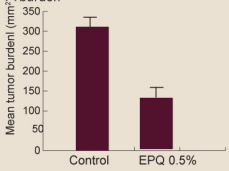
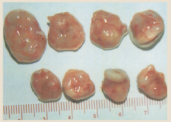
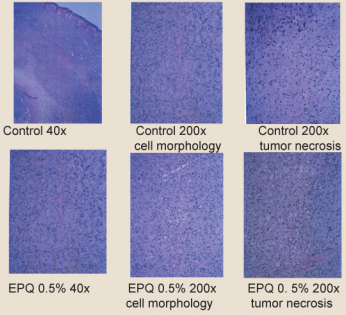


Figure 2- Representative tumors from groups: gross photographs



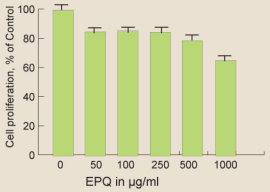
2. Histologically, the tumors from both groups were irregularly round, skin subcutaneous masses, consistent with clear cell ovarian carcinoma. Areas of tumor necrosis ranged from 50-80% of tumor mass in the Control group compared to 20-70% in the EPQ group.

Figure 3 - Histopathology of tumors



3. EPQ exhibited dose-dependent inhibition of ovarian ES-2 cell proliferation *in vitro* with 35% ($p<0.0001$) decrease at 1000 µg/ml EPQ, compared to the Control (Figure 4).

Figure 4 - Effect of EPQ on ES-2 cell proliferation



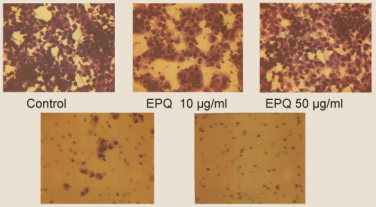
4. Zymography only demonstrated MMP-2 secretion by normal and PMA-treated ES-2 cells. EPQ inhibited MMP-2 secretion in a dose-dependent fashion with near total inhibition at 500 and 1000 µg/ml, as shown in Figure 5.

Figure 5A Normal ES-2 cells *Figure 5B - PMA-treated ES-2 cells*

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

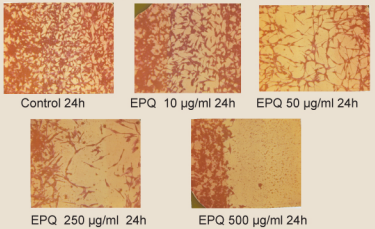
5. EPQ significantly inhibited ES-2 cell invasion through Matrigel in a dose-dependent manner, with total block at 500 µg/ml, as shown in Figure 6.

Figure 6 - Effect of EPQ on ES-2 cell invasion: photomicrographs



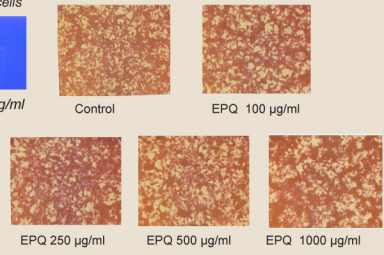
6. EPQ reduced cell migration in a dose-dependent manner, with complete block of ES-2 cells at 500 µg/ml. Photomicrographs of the results for the scratch tests for ES-2 cells are shown in Figure 7.

Figure 7- Effect of EPQ on ES-2 cell migration: scratch test



7. H&E staining showed no morphological changes below EPQ 1000 µg/ml, as shown in Figure 8.

Figure 8- Effect of EPQ on ES-2 morphology:H&E



4.Conclusion

Our studies demonstrated that the mixture of the non-toxic components of EPQ significantly inhibited the growth and tumor burden of ovarian cancer cell line ES-2 *in vivo*. In addition, invasive parameters, such as ES-2 cell line MMP-2 secretion, migration and invasion were significantly inhibited by EPQ *in vitro*. These findings suggest potential of EPQ in treatment of ovarian cancer.