A unique nutrient mixture suppresses ovarian cancer growth of A-2780 by inhibiting invasion and MMP-9 secretion

M.W. Roomi*, A. Niedzwiecki and M. Rath
Dr. Rath Research Institute, 1260 Memorex Drive, Santa Clara, CA 95050

Introduction
Ovarian cancer is the deadliest gynecological malignancy in women, and the fifth leading cause of death. The American Cancer Society estimated that it would claim 14,250 lives in 2013. Despite the advances made in chemotherapy and surgery, the average time of clinical remission is approximately 2 years and the 5-year survival rate is 45%. Thus, there is an urgent need for the development of a novel therapeutic approach to ovarian cancer treatment.

Objective
We investigated the effect of a unique nutrient mixture (EPQ®) containing acetic acid, lysine, proline, green tea extract and quercetin on human ovarian cancer cell A-2780 in vivo and in vitro.

Materials and Methods
In vivo
1. Athymic female nude mice (n=12) were inoculated i.p. with 2x10⁶ cells in 0.1ml PBS and randomly divided into two groups. Group A (n=6) was fed a regular diet and group B (n=6) a regular diet supplemented with 0.5% EPQ.
2. Four weeks later, the mice were sacrificed and tumors that developed in the ovaries were excised, weighed and processed for histology.

In vitro
1. A-2780 cells were cultured in Dulbecco modified Eagle medium supplemented with 10% FBS and antibiotics. At near confluence, cells were treated with EPQ in triplicate at concentrations between 0-1000 μg/ml. 
2. Cell proliferation was measured by MTT assay, MMP-9 secretion by gelatinase zymography, invasion through Matrigel, and morphology by H&E staining.

Composition of Nutrient Mixture (NM)
- Vitamin C as ascorbic acid and ascorbic acid 700 mg
- Mg, Ca and phosphate ascorbate (200 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

Results
1. All control mice developed large ovarian tumors, whereas the EPQ group developed no tumors in 5 of 6 mice and a small tumor in the 6th mouse. EPQ inhibited mean tumor weight by 87% (p<0.0001), as shown in Figure 1A. Control group mice showed metastasis to lungs in 6 out of 6 mice, while there was no lung metastasis in the EPQ group (Figure 1C).

2. Control group histopathology
   Examination of sections of ovaries, cervix, vagina and ovaries of EPQ group of mice, showed mild to moderate multiple small glandular cysts in the uterus. One ovary was totally replaced by a large tumor composed of irregularly-round cells with indistinct cell borders and irregularly rounded nuclei, possibly of luteal or granulosa cell origin. Mitotic figures range from 4-5 per high powered field. Multiple foci of necrosis involved about 40% of the tumor mass (Figure 3).

3. EPQ group histopathology
   Examination of sections of ovaries, cervix, vagina and ovaries of EPQ group of mice, showed mild to moderate multiple small glandular cysts in the uterus. One ovary was totally replaced by a large tumor composed of irregularly-round cells with indistinct cell borders and irregularly rounded nuclei, possibly of luteal or granulosa cell origin. Mitotic figures range from 4-5 per high powered field. Multiple foci of necrosis involved about 40% of the tumor mass (Figure 3).

4. In vitro, EPQ exhibited dose dependent inhibition of cell proliferation (p=0.0001), with 65% toxicity over the control at 250 μg/ml, 73% at 500 μg/ml concentrations, and 80% at 1000 μg/ml, as shown in Figure 4.

Conclusions
These results suggest that EPQ has therapeutic potential in treatment of ovarian cancer by significantly suppressing tumor growth and by inhibiting MMP-9 secretion and invasion of ovarian cancer A-2780 cells.