Am J Cancer Res 2017;7(6):1322-1336
www.ajcr.us /ISSN:2156-6976/ajcr0046448

Original Article

Lycopene reduces ovarian tumor growth and intraperitoneal metastatic load

Nina Pauline Holzapfel[1], Ali Shokoohmand[1,2], Ferdinand Wagner[1,3], Marietta Landgraf[1], Simon Champ[4], Boris Michael Holzapfel[1,5], Judith Ann Clements[1,2], Dietmar Werner Hutmacher[1,2,6,7], Daniela Loessner[1]

[1]Queensland University of Technology (QUT), 60 Musk Avenue, Kelvin Grove, QLD 4059, Brisbane, Australia; [2]Australian Prostate Cancer Research Centre-Queensland, Translational Research Institute, 37 Kent Street, Woolloongabba, QLD 4102, Brisbane, Australia; [3]Department of Pediatric Surgery, Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University Munich, Lindwurmstr. 4, 80337 Munich, Germany; [4]Human Nutrition, BASF SE, G-ENH/MB, 68623 Lampertheim, Germany; [5]Orthopaedic Center for Musculoskeletal Research, University of Wuerzburg, Koenig-Ludwig Haus, Brettreichstr. 11, 97074 Wuerzburg, Germany; [6]George W Woodruff School of Mechanical Engineering, Georgia Institute of Technology, 801 Ferst Drive Northwest, Atlanta 30332, GA, USA; [7]Institute for Advanced Study, Technical University of Munich, Lichtenbergstr. 2a, 85748 Munich, Germany

Received December 12, 2016; Accepted December 15, 2016; Epub June 1, 2017; Published June 15, 2017

Abstract: Mutagens like oxidants cause lesions in the DNA of ovarian and fallopian tube epithelial cells, resulting in neoplastic transformation. Reduced exposure of surface epithelia to oxidative stress may prevent the onset or reduce the growth of ovarian cancer. Lycopene is well-known for its excellent antioxidant properties. In this study, the potential of lycopene in the prevention and treatment of ovarian cancer was investigated using an intraperitoneal animal model. Lycopene prevention significantly reduced the metastatic load of ovarian cancer-bearing mice, whereas treatment of already established ovarian tumors with lycopene significantly diminished the tumor burden. Lycopene treatment synergistically enhanced anti-tumorigenic effects of paclitaxel and carboplatin. Immunostaining of tumor and metastatic tissues for Ki67 revealed that lycopene reduced the number of proliferating cancer cells. Lycopene decreased the expression of the ovarian cancer biomarker, CA125. The anti-metastatic and anti-proliferative effects were accompanied by down-regulated expression of *ITGA5*, *ITGB1*, *MMP9*, *FAK*, *ILK* and EMT markers, decreased protein expression of integrin α5 and reduced activation of MAPK. These findings indicate that lycopene interferes with mechanisms involved in the development and progression of ovarian cancer and that its preventive and therapeutic use, combined with chemotherapeutics, reduces the tumor and metastatic burden of ovarian cancer *in vivo*.

Keywords: Antioxidant, ovarian cancer, lycopene, humanized animal model, hydrogels

Introduction

Ovarian cancer is the fifth leading cause of cancer death in women, with an estimate of 22,280 newly diagnosed cases and 14,240 deaths in the United States in 2016 [1], and it is the gynecological malignancy associated with the worst survival rates [2]. About 75% of affected women are diagnosed at a late stage of the disease, when the cancer has metastasized into the peritoneal cavity, which is one of the reasons for the high mortality rates associated with this cancer [2]. The main risk factors for developing ovarian cancer are a positive family history, age and infertility [3]. During the peri-ovulatory period, mutagens, such as oxidants and inflammatory markers, are generated and in contact with ovarian and fallopian tube epithelial cells. As a result, these cells can undergo DNA damage [4]. Oxidative stress develops from an imbalance between the generation and detoxification of reactive oxygen species [5]. Reduction of oxidative stress may therefore be considered as a possible preventive mechanism for ovarian cancer [6].

A wide range of antioxidants, present in a variety of fruits and vegetables, have been shown to minimize DNA damage by inactivating free radicals and are investigated for their potential

as preventive agents for different types of cancer [7]. Carotenoids, selenium, green tea and alpha-tocopherol are well-known antioxidants for their potential cancer-preventive properties [8, 9]. The most powerful carotenoid with antioxidant capacity is lycopene [10]. Lycopene is naturally occurring in red carotenoid pigments found in tomatoes and processed tomato products and has received increasing attention within the scientific community, as well as in the public perception, regarding its potential to decrease the risk of developing prostate, breast and ovarian cancers [10, 11]. However, the existing data that suggest a positive correlation between the dietary or supplemental intake of lycopene and a lower risk of ovarian cancer are rather inconsistent [12].

The aims of this study were to investigate lycopene's (i) preventive and therapeutic effects in ovarian cancer development and progression, (ii) ability to enhance the efficacy of clinically used chemotherapeutics and (iii) interacting molecular factors in a bioengineered disease model of ovarian cancer.

Materials and methods

Reagents

Lycopene and the placebo were provided by BASF (Ludwigshafen, Germany) as a water-dispersible beadlet formulation, containing 10% lycopene in a matrix of carbohydrates and no lycopene for the placebo respectively. Polyethylene glycol-based hydrogels (RGD-functionalized, proteolytic degradable; QGel, Switzerland) served as cancer cell delivery vehicles for animal experiments. Cell culture and qRT-PCR reagents were purchased from Life Technologies (VIC, Australia). Paclitaxel and carboplatin were from Sigma-Aldrich (NSW, Australia). Immunoblotting reagents included pre-made NuPAGE gradient SDS-PAGE gels (4-12%; Life Technologies), phosphatase inhibitor (Life Technologies), complete protease inhibitor (Roche Diagnostics, NSW, Australia), Pierce™ Coomassie (Bradford) protein assay (Life Technologies), BioTrace NT nitrocellulose membranes (Pall Corporation, NSW, Australia), enhanced chemiluminescence (ECL) reagents (GE Healthcare, NSW, Australia), X-ray films (Fujifilm, NSW, Australia) and the following antibodies: anti-integrin α5 (Merck Millipore, VIC, Australia), anti-integrin β1 (Merck Millipore), anti-phospho-p44/42 MAPK (ERK1/2, Thr202/Tyr204, #E10; Cell Signaling Technology, USA), anti-p44/42 MAPK (ERK1/2; Cell Signaling Technology), anti-β-tubulin (Cell Signaling Technology) and HRP-linked anti-mouse/rabbit IgG (Cell Signaling Technology). Other items included: D-luciferin (Caliper Life Sciences, USA), CA125 and MMP9 ELISAs (R&D Systems, USA). Immunohistochemistry reagents were purchased from Dako (VIC, Australia), and the following human-specific antibodies were used: NuMA (Abcam, VIC, Australia), Ki67 (#MIB-1; Dako) and anti-integrin α5β1 (#JBS5; Merck Millipore).

Cell culture

Ovarian cancer OV-MZ-6 cells were established from malignant ascites drained from a patient suffering from advanced serous cystadeno-carcinoma and were maintained as reported [13]. For animal experiments, OV-MZ-6 cells were transfected with a lentiviral luciferase expression system as described [14]. Luciferase-transfected OV-MZ-6 cells were then encapsulated within hydrogels (1.6×10^4 cells/20 µl hydrogel) and cultured for 4 days prior to implantation into animals.

Preparation of lycopene dispersions

For cell experiments, lycopene was dispersed in sterile PBS resulting in a stock concentration of 1 mM. Treatment dispersions were prepared fresh for each experiment from the stock concentration in culture medium to obtain final concentrations of 2.0 and 5.0 µM lycopene. For control treatments, a stock concentration of the placebo was prepared and diluted in culture medium accordingly. For animal experiments, lycopene and the placebo were diluted in ultrapure DNase/RNase-free water to a final concentration of 0.75 mg/ml.

Western blot analysis

OV-MZ-6 cells were cultured in 6-well plates (1×10^5 cells/well) for 72 hrs prior to treatment with 2.0 and 5.0 µM lycopene or placebo in culture medium supplemented with 1% FBS for 10 min, 1, 12 and 24 hrs. For protein extraction, cells were washed and lysed in radioimmunoprecipitation assay buffer (150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, ph 8.0), containing 10% phosphatase and complete protease inhibitors.

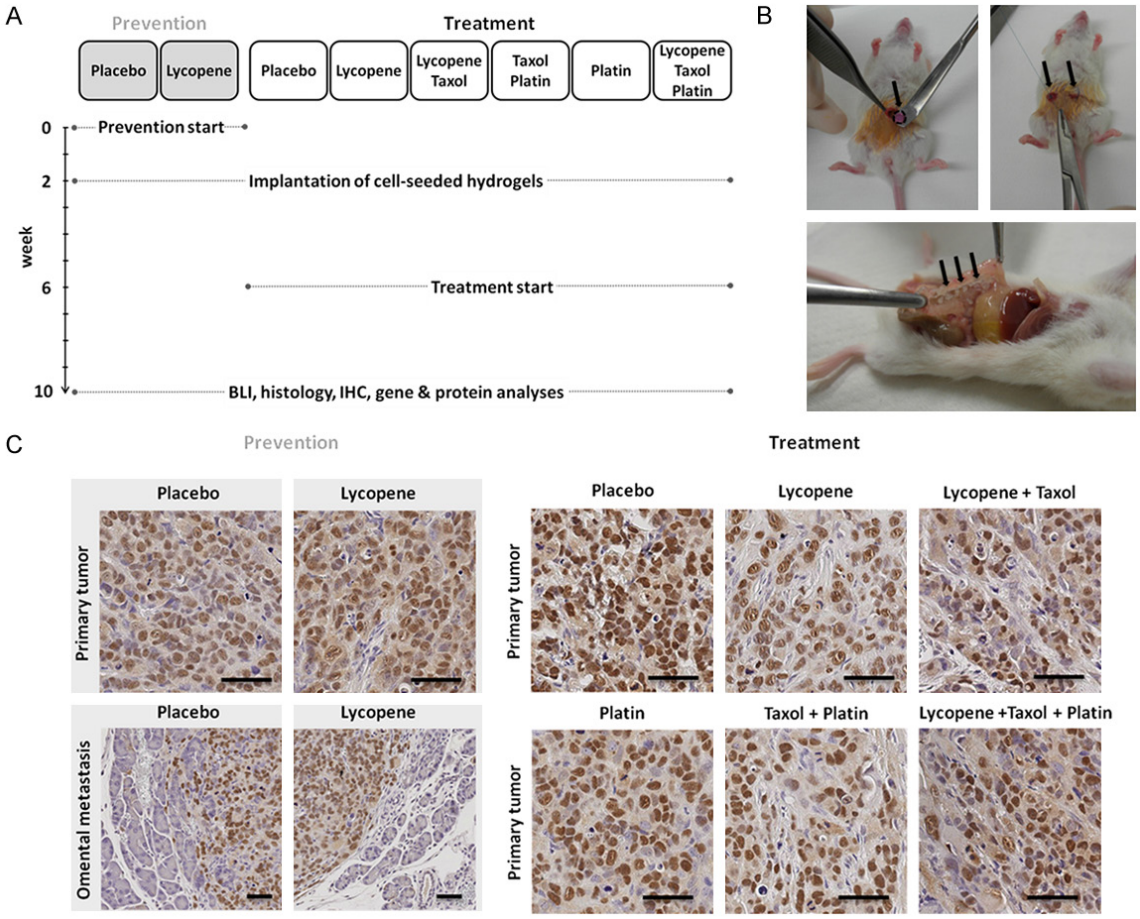


Figure 1. Experimental outline and treatment regimen. A. Prevention started 2 weeks prior to implantation of cell-seeded hydrogels into NOD/SCID mice by daily lycopene and placebo administration via oral gavage over 10 weeks. Treatment started 4 weeks post implantation using placebo, lycopene and carboplatin (platin) only or in combination with paclitaxel (taxol) or as a triple treatment regimen over 4 weeks. Taxol and platin were given twice per week in an alternating manner via intraperitoneal injections every fortnight. B. Implantation of cell-seeded hydrogels into the peritoneal cavity was performed via abdominal incision parallel to the longitudinal body axis. Hydrogels were placed adjacent to the ovarian fat pad on each side (top panel, arrows). During necropsy, omental metastases were evident upon placebo and lycopene prevention and treatment (lower panel, arrows). C. Immunohistochemistry using a human-specific antibody against NuMA confirmed that tumors and omental metastases were of human origin. Scale bars, 50 µm.

Lysates were centrifuged for 15 min at 14,000 g, the supernatants collected, and protein concentrations determined using a Pierce™ Coomassie (Bradford) protein assay. SDS-PAGE-resolved total protein samples (20 µg) were transferred onto nitrocellulose membranes and probed with anti-integrin α5, anti-integrin β1, anti-phospho-p44/42 MAPK and anti-p44/42 MAPK (1:1,000). After washing, membranes were incubated with HRP-linked anti-mouse/rabbit IgG (1:5,000). Total protein levels were validated for equal loading using anti-β-tubulin (1:4,000). Reactive proteins were visualized via ECL, signals transferred onto X-ray films and developed with an Agfa CP-1000 film processor (Superior Radiographics, USA).

Intraperitoneal animal model

Animal experiments were performed in compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and approved by the University Animal Ethics Committee. Surgery using 6-week old female NOD/SCID mice was performed as described [14]. Equal distribution of cells encapsulated within hydrogels prior to implantation was confirmed via bioluminescence imaging (BLI; $3.15 \times 10^6 \pm 6.39 \times 10^5$ p/s/cm^2/

Table 1. Sequences of gene-specific primers used for qRT-PCR, performed with an annealing temperature of 60°C, and their respective product size

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)
ITGA5	CATTTCCGAGTCTGGGCCAA	TGGAGGCTTGAGCTGAGCTT	324
ITGB1	AGGTGGTTTCGATGCCATCAT	AAGTGAAACCCGGCATCTGTG	105
MMP9	TCGTGGTTCCAACTCGGTTT	GCGGCCCTCGAAGATGA	71
FAK	GCGCTGGCTGGAAAAAGAGGAA	TCGGTGGGTGCTGGCTGGTAGG	475
ILK	CCAATGTCCTGGTCGCATGTA	CGTGTCACCAGTTCCCACAGA	132
TWIST	GGAGTCCGCAGTCTTACGAG	TCTGGAGGACCTGGTAGAGG	201
ZEB2	TTCCTGGGCTACGACCATAC	TGTGCTCCATCAAGCAATTC	160
SNAI1	CCTCCCTGTCAGATGAGGAC	CCAGGCTGAGGTATTCCTTG	234
SNAI2	GGGGAGAAGCCTTTTTCTTG	TCCTCATGTTTGTGCAGGAG	158
FOXC2	GCCTAAGGACCTGGTGAAGC	TTGACGAAGCACTCGTTGAG	198
FN1	CAGTGGGAGACCTCGAGAAG	TCCCTCGGAACATCAGAAAC	168
TGFB1	CGTGGAGGGGAAATTGAGGG	CCGGTAGTGAACCCGTTGATG	98
TGFB2	ACAGCACCAGGGACTTGCTCCA	TGGGCGGGATGGCATTTTCGG	147
TGFBR1	TGTTGGTACCCAAGGAAAGC	CACTCTGTGGTTTGGAGCAA	160
SMAD4	AGGATCAGTAGGTGGAATAGC	TGCATAAGCGACGAAGG	83
18S	GATCCATTGGAGGGCAAGTCT	CCAAGATCCAACTACGAGCTTTTT	103

sr; n = 10 hydrogels) using a live animal imaging machine (IVIS® Spectrum 200, Perkin Elmer, VIC, Australia; Living Image® software v.4.3.1). To determine preventative effects of lycopene on tumor growth and metastasis, prevention started 2 weeks prior to implantation of cell-seeded hydrogels into animals (n = 5). To examine therapeutic effects of lycopene on tumor growth and metastasis, treatment started 4 weeks after surgery (n = 5; **Figure 1A**). Hydrogels were implanted adjacent to both ovaries, and abdominal muscles and the skin were sutured separately (**Figure 1B**, upper panel). Animals in the prevention (lycopene and placebo) and treatment groups (lycopene, placebo, paclitaxel (taxol), lycopene + taxol, carboplatin (platin), lycopene + platin, taxol + platin and lycopene + taxol + platin) were monitored via regular weight measurements and BLI. Lycopene and placebo (15 mg/kg each) were administered daily via oral gavage. Taxol (10 mg/kg) and platin (15 mg/kg) were administered twice per week in an alternating manner via intraperitoneal injections every fortnight. Eight weeks post surgery, animals were sacrificed, and tumor and metastatic tissues were removed, weighed and processed for qRT-PCR and immunohistochemical analysis. To distinguish between tumor load and metastatic load, animals were imaged by *ex vivo* BLI prior to and post removal of the primary tumors. Serum and ascites were collected and pooled for each group.

Quantitative RT-PCR

Tissues were dispersed with a tissue homogenizer, and RNA extracted using Trizol® reagent following manufacturer's instructions. RNA quality was determined with a Nanodrop® ND-1000 spectrophotometer (Life Technologies), and samples with $A_{260nm/230nm}$ = 2.05-2.15 were synthesized into cDNA using a Superscript™ III first-strand synthesis supermix following manufacturer's instructions. Quantitative RT-PCR was performed with SYBR® green chemistry using an ABI Prism® 7500 sequence detection system (Applied Biosystems, VIC, Australia) as reported [14]. Gene-specific primers are listed in **Table 1**. The cycle threshold (C_t) value for each gene was determined and normalized to *18S* expression levels and compared to the control ($\Delta\Delta C_t$). Relative gene expression between groups was calculated using the 2^(-$\Delta\Delta C_t$) method.

ELISA

To quantify the concentrations of human CA125 and MMP9 in mouse serum and ascites, human-specific CA125 and MMP9 ELISAs were conducted following manufacturer's instructions. Samples were diluted 2-fold for CA125 and 40-fold for MMP9 detection.

Immunohistochemistry

Immunohistochemical analysis was performed on serial paraformaldehyde-fixed paraffin-embedded sections (5 μm). Sections were deparaffinized in xylene and rehydrated in dilutions of ethanol and water. Antigen retrieval was performed in a high pH buffer (pH 9) or citrate buffer (pH 6) at 95°C for up to 10 min. Then, sections were treated with 3% H_2O_2 and blocked with 2% BSA/PBS. Antibodies were applied in 2% BSA/PBS (NuMA 1:100; Ki67

1:75; integrin α5β1 1:200; phospho-p44/42 MAPK 1:100; p44/42 MAPK 1:100; vimentin 1:2,000). After washing, sections were incubated with EnVision + Dual Link System-HRP, followed by 3,3'-diaminobenzidine and Mayer's hematoxylin. Sections were imaged with an automatic slide scanner (Leica, NSW, Australia), with a 40× magnification, and archived on a digital image hub. Staining was quantified using ImageJ (mean ± SEM; n = 5; NIH, USA).

Statistical analysis

GraphPad Prism® software (v.6) was used for statistical analyses. Data were presented as mean values ± standard errors of three biological replicate experiments. Differences between treatment regimen and the control were analyzed using One-way ANOVA and Student's t-test (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$).

Results

A humanized intraperitoneal animal model based on a bioengineering platform

Our group has developed a bioengineered 3D cancer model that is based on hydrogels as human cancer cell delivery vehicle when placed intraperitoneally into a murine host [14]. In this study, our humanized animal model was used to test the preventive and therapeutic effects of lycopene in ovarian cancer (**Figure 1A**, **1B**). To confirm that the tumor and metastatic loads observed in NOD/SCID mice were of human origin, immunohistochemical analysis of tumor and metastatic tissues was performed using a human-specific antibody against the nuclear mitotic apparatus protein 1 (NuMA) [15]. In both prevention and treatment regimen, neoplastic tissues stained positive for NuMA as evident by brown nuclei (**Figure 1C**). Metastases colonizing the omentum were also stained positive for NuMA (**Figure 1C**).

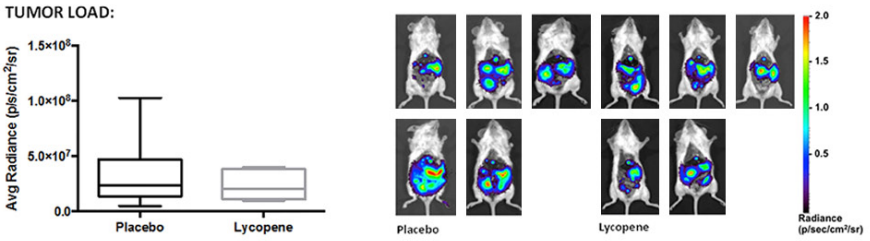
Lycopene prevention reduced intraperitoneal metastatic load, cancer-related factors and CA125

To examine the preventive effects of lycopene on tumor growth and metastasis, animals received lycopene and placebo 2 weeks prior to implantation of cell-seeded hydrogels. Upon lycopene prevention, the tumor load resulted in an average radiance of $2.34 \times 10^7 \pm 4.51 \times 10^6$ p/s/cm^2/sr, while administration of the placebo resulted in an average radiance of $3.37 \times 10^7 \pm 9.10 \times 10^6$ p/s/cm^2/sr upon BLI analysis (**Figure 2A**). This indicates a trend towards a preventive benefit of lycopene, although the difference to the placebo was not significant ($P = 0.36$). Strikingly, the metastatic load determined by BLI analysis after removal of the primary tumors was significantly lower upon lycopene prevention ($4.01 \times 10^6 \pm 9.90 \times 10^5$ p/s/cm^2/sr; $P = 0.03$) compared to the placebo ($1.60 \times 10^7 \pm 5.26 \times 10^6$ p/s/cm^2/sr; **Figure 2B**). This was a decrease of an order of a magnitude, unlikely arising from different primary tumor load. Immunohistochemical staining of human-specific Ki67, a cell proliferation marker [16], in tumor tissue revealed more proliferating cells upon placebo prevention compared to lycopene as indicated by the fraction of brown nuclei and quantitative image analysis (**Figure 2C**). This effect was also visible in metastatic tissue, which exhibited a stronger staining of Ki67 upon placebo prevention compared to lycopene prevention.

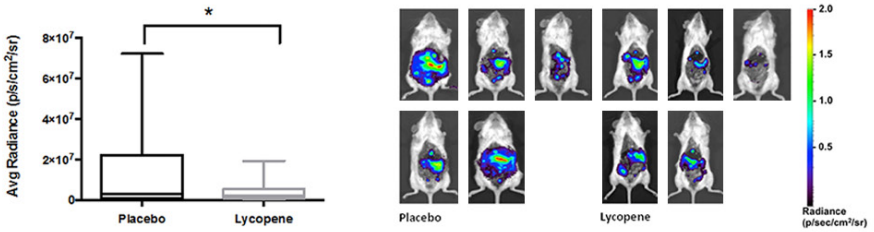
To identify lycopene-responsive factors, the expression of ovarian cancer-related genes was analyzed in tumor and metastatic tissues [17-19]. The gene expression of integrin α5 (*ITGA5*), integrin β1 (*ITGB1*), integrin-linked kinase (*ILK*) and focal adhesion kinase (*FAK*) were significantly decreased in tumor tissue upon lycopene prevention compared to the placebo (**Figure 2D**, left panel). Matrix metalloproteinase 9 (*MMP9*) expression was also decreased in tumor tissue. Similar results were observed in metastatic tissue, with a further down-regulation of *ITGA5* and *MMP9* (**Figure 2D**, right panel).

To further delineate the preventative impact of lycopene on the production of the integrin α5β1 heterodimer and MMP9, two key players in ovarian cancer cell invasion and metastasis [20, 21], immunohistochemical analysis of tumor and metastatic tissues using a human-specific antibody against integrin α5β1 and ELISA were performed. Staining of integrin α5β1 in tumor tissue was weaker upon lycopene prevention compared to the placebo (**Figure 2E**), while metastatic tissue revealed a more intense staining upon lycopene prevention, which was confirmed by quantitative image analysis. Levels of human-derived MMP9 in serum and ascites collected from the animals were reduced upon lycopene prevention compared to the placebo (**Figure 2F**, left panel).

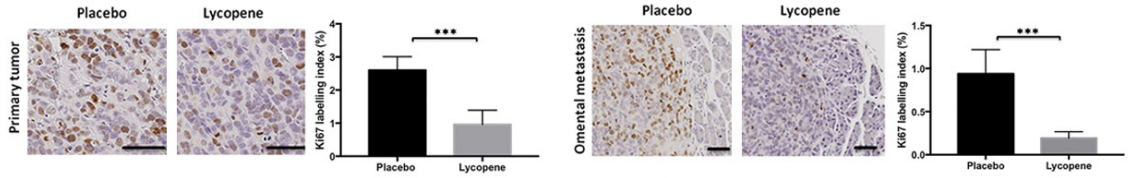
A



B



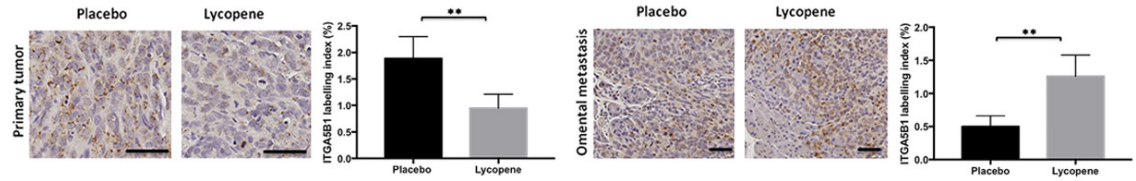
C



D

	PRIMARY TUMOR:			METASTASES:		
Gene	Placebo	Lycopene	P-value	Placebo	Lycopene	P-value
ITGA5	1 ± 0.08	0.56 ± 0.02	0.01	1 ± 0.03	0.20 ± 0.01	< 0.0001
ITGB1	1 ± 0.03	0.25 ± 0.00	< 0.0001	1 ± 0.01	0.26 ± 0.03	< 0.0001
MMP9	1 ± 0.03	0.73 ± 0.04	0.23	1 ± 0.02	0.35 ± 0.01	< 0.0001
ILK	1 ± 0.02	0.25 ± 0.00	< 0.0001	1 ± 0.02	0.76 ± 0.01	0.0001
FAK	1 ± 0.10	0.26 ± 0.00	0.0002	1 ± 0.06	0.78 ± 0.03	0.02

E



F

	Human MMP9 (ng/ml)			Human CA125 (U/ml)		
	Placebo	Lycopene	P-value	Placebo	Lycopene	P-value
Serum	83.12 ± 1.57	80.11 ± 1.08	0.19	10.08 ± 0.18	8.77 ± 0.27	0.02
Ascites	75.04 ± 4.03	70.84 ± 1.20	0.38	6.99 ± 0.15	5.68 ± 0.15	0.003

Figure 2. Effects of lycopene prevention. A. Tumor load was lower upon lycopene prevention compared to the placebo (average radiance, mean ± SEM; n = 5). B. Metastatic load was significantly reduced upon lycopene prevention compared to the placebo (average radiance, mean ± SEM; n = 5; $P = 0.03$). C. Immunohistochemical staining of Ki67 was more intense in tumor than metastatic tissues, with less positively stained cells upon lycopene prevention (mean ± SEM; n = 5; $P \leq 0.001$). Scale bars, 50 μm. D. Expression of *ITGA5*, *ITGB1*, *ILK* and *FAK* was significantly down-regulated in tumor and metastatic tissues upon lycopene prevention. Expression of *MMP9* was significantly reduced in metastatic tissue. Values are normalized to placebo controls. E. Immunohistochemical staining of integrin α5β1 was weaker in tumor tissue and more intense in metastatic tissue upon lycopene prevention (mean ± SEM; n = 5; $P \leq 0.01$). Scale bars, 50 μm. F. Levels of MMP9 in serum and ascites were reduced upon lycopene prevention (left panel). Levels of CA125 in serum and ascites were significantly lower upon lycopene prevention compared to the placebo (right panel).

Table 2. Gene expression of key EMT markers was significantly down-regulated in metastatic tissue upon lycopene prevention (values are normalized to placebo controls)

Gene	Placebo	Lycopene	*P*-value
TWIST	1 ± 0.03	0.17 ± 0.04	0.0075
ZEB2	1 ± 0.07	0.19 ± 0.01	< 0.0001
Snail (SNAI1)	1 ± 0.04	0.31 ± 0.02	< 0.0001
Slug (SNAI2)	1 ± 0.13	0.18 ± 0.03	0.013
FOXC2	1 ± 0.04	0.65 ± 0.03	< 0.0001
FN1	1 ± 0.04	0.39 ± 0.02	< 0.0001
TGFB1	1 ± 0.06	0.19 ± 0.01	< 0.0001
TGFB2	1 ± 0.03	0.36 ± 0.03	< 0.0001
TGFBR1	1 ± 0.04	0.43 ± 0.01	< 0.0001
SMAD4	1 ± 0.06	0.24 ± 0.01	< 0.0001

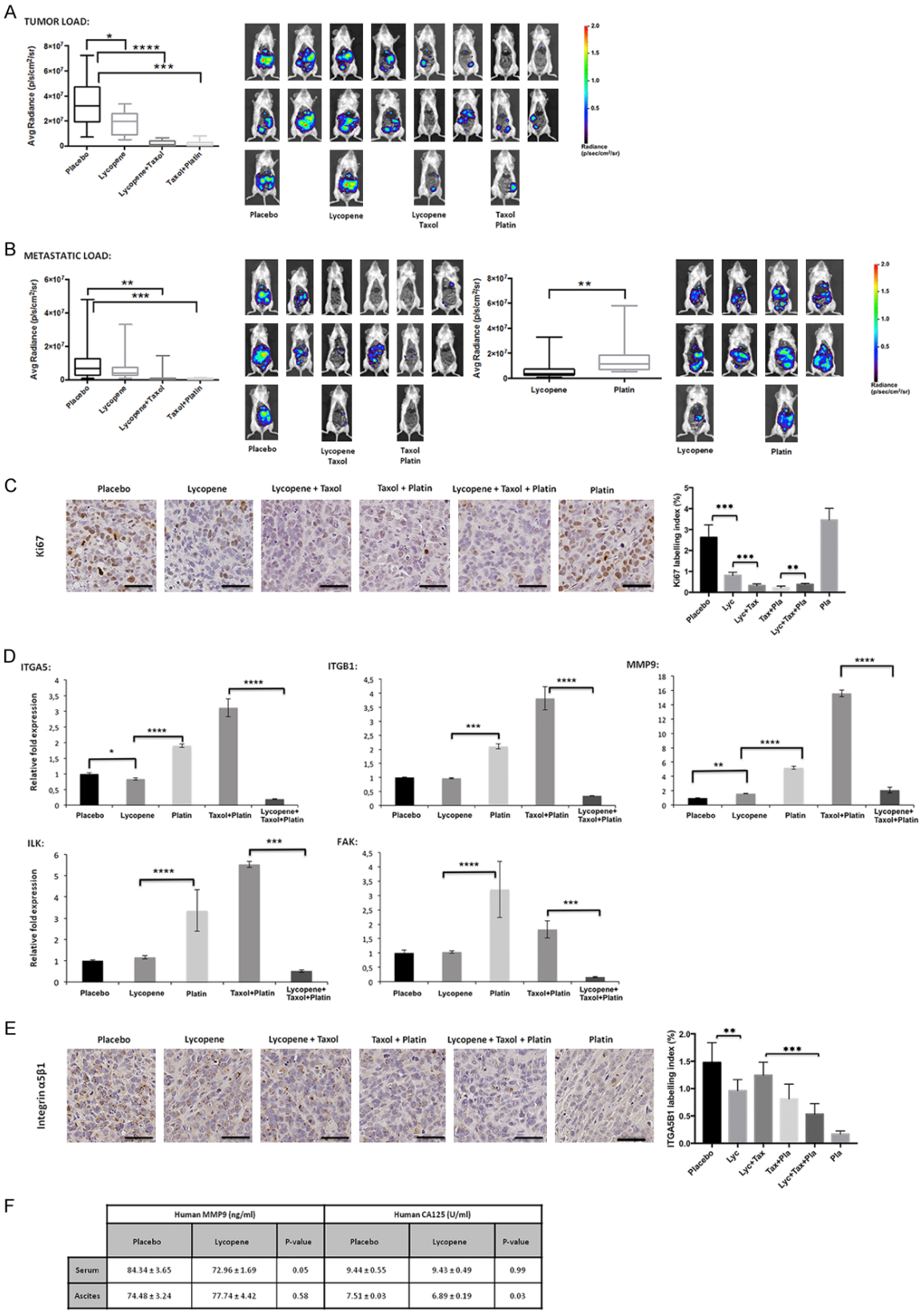
A similar result was obtained for the clinically used ovarian cancer biomarker, CA125 [22]. Lycopene prevention led to significantly reduced levels of human-specific CA125 in serum and ascites compared to the placebo (**Figure 2F**, right panel).

Lastly, it was sought to determine whether lycopene is also capable to hinder intraperitoneal tumor progression. Thus, the expression of well-known epithelial to mesenchymal transition (EMT)-related genes in metastatic tissue was analyzed [23]. Strikingly, the expression of all examined EMT markers was significantly down-regulated in animals receiving lycopene prevention compared to the placebo (**Table 2**). Overall, preventive administration of lycopene interfered with tumor metastasis, metastasis-mediating factors and CA125.

Lycopene synergistically enhanced anti-tumorigenic effects of chemotherapeutics

To examine the potential effect of lycopene as a cancer treatment individually and synergistically to clinically used chemotherapeutics, animals received daily lycopene or placebo 4 weeks post implantation of cell-seeded hydrogels in combination with taxol or platin or both. Lycopene treatment of tumor-bearing mice significantly reduced the tumor load ($1.88 \times 10^7 \pm 3.13 \times 10^6$ p/s/cm^2/sr; $P = 0.03$) compared to the placebo ($3.52 \times 10^7 \pm 6.21 \times 10^6$ p/s/cm^2/sr) upon BLI analysis (**Figure 3A**). The lycopene + taxol combination treatment ($2.25 \times 10^6 \pm 8.00 \times 10^5$ p/s/cm^2/sr; $P < 0.0001$) was as effective in reducing the tumor load as the taxol + platin combination ($1.87 \times 10^6 \pm 1.01 \times 10^6$ p/s/cm^2/sr; $P = 0.0002$) compared to the placebo (**Figure 3A**). The lycopene + taxol combination treatment ($1.42 \times 10^6 \pm 8.90 \times 10^5$ p/s/cm^2/sr; $P = 0.005$) was also as effective as the taxol + platin combination in reducing the metastatic load ($4.05 \times 10^5 \pm 8.90 \times 10^4$ p/s/cm^2/sr; $P = 0.0008$) compared to the placebo ($9.82 \times 10^6 \pm 1.12 \times 10^7$ p/s/cm^2/sr; **Figure 3B**, left panel). There were no macroscopically or histologically detectable omental metastases and ascites in animals treated with lycopene + taxol, taxol + platin and lycopene + taxol + platin. Taxol treatment reduced tumor and metastatic loads (tumor load: $5.55 \times 10^6 \pm 8.80 \times 10^6$ p/s/cm^2/sr; metastatic load: $2.36 \times 10^6 \pm 2.35 \times 10^6$ p/s/cm^2/sr; data not shown), while lycopene + platin combination treatment (tumor load: $2.88 \times 10^7 \pm 2.99 \times 10^7$ p/s/cm^2/sr; metastatic load: $1.30 \times 10^7 \pm 1.15 \times 10^7$ p/s/cm^2/sr; data not shown) did not show any differences compared to platin treatment (tumor load: $2.86 \times 10^7 \pm 2.25 \times 10^7$ p/s/cm^2/sr; metastatic load: $1.62 \times 10^7 \pm 1.24 \times 10^7$ p/s/cm^2/sr), indicating that this tumor model might be less sensitive to platin. Moreover, lycopene administered as a single agent treatment was more effective in reducing metastatic growth ($6.52 \times 10^6 \pm 7.47 \times 10^6$ p/s/cm^2/sr; $P = 0.001$) than platin only ($1.62 \times 10^7 \pm 1.24 \times 10^7$ p/s/cm^2/sr; **Figure 3B**, right panel). Immunohistochemical analysis of Ki67 showed the strongest staining in tumor tissue upon placebo and platin treatment and a less intense staining upon lycopene treatment, indicating a reduced cell proliferation upon lycopene treatment. The different combination regimen, lycopene + taxol, taxol + platin and lycopene + taxol + platin, showed a similar Ki67 staining intensity, with all three treatments showing a lower staining intensity than lycopene only as indicated by quantitative image analysis (**Figure 3C**). These results demonstrate the anti-tumorigenic effects of lycopene. In combination treatment with taxol, lycopene was as effective as platin and had synergistic effects in reducing the tumor and metastatic burden. Lycopene might be useful to reduce the development of intraperitoneal metastases that are less sensitive to platin.

Next, the impact of lycopene treatment on the expression of ovarian cancer-related genes was analyzed. Lycopene treatment significantly



F

	Human MMP9 (ng/ml)			Human CA125 (U/ml)		
	Placebo	Lycopene	P-value	Placebo	Lycopene	P-value
Serum	84.34 ± 3.65	72.96 ± 1.69	0.05	9.44 ± 0.55	9.43 ± 0.49	0.99
Ascites	74.48 ± 3.24	77.74 ± 4.42	0.58	7.51 ± 0.03	6.89 ± 0.19	0.03

Figure 3. Effects of lycopene treatment ± chemotherapeutics. A. Tumor load was significantly reduced upon lycopene ($P = 0.03$), lycopene + taxol ($P < 0.0001$) and taxol + platin ($P = 0.0002$) regimen compared to the placebo (average radiance, mean ± SEM; n = 5). B. Metastatic load was significantly reduced upon lycopene + taxol ($P = 0.005$) and taxol + platin ($P = 0.0008$) regimen compared to the placebo (average radiance, mean ± SEM; n = 5). Lycopene reduced the metastatic load significantly more than platin ($P = 0.001$). C. Immunohistochemical staining of Ki67 in tumor tissue was most intense upon placebo and platin treatment, with all combination regimen having less positively stained cells than lycopene only (mean ± SEM; n = 5; $P \leq 0.01$). Scale bars, 50 µm. D. Lycopene significantly reduced *ITGA5* ($P = 0.02$) and increased *MMP9* ($P = 0.001$) expression. Expression of *ITGA5* ($P < 0.0001$), *ITGB1* ($P = 0.0006$), *MMP9* ($P < 0.0001$), *ILK* ($P < 0.0001$) and *FAK* ($P < 0.0001$) were significantly induced upon platin compared to lycopene. Expression of *ITGA5* ($P < 0.0001$), *ITGB1* ($P < 0.0001$), *MMP9* ($P < 0.0001$), *FAK* ($P = 0.0002$) and *ILK* ($P = 0.0002$) were significantly reduced upon lycopene + taxol + platin compared to taxol + platin. E. Immunohistochemical staining of integrin α5β1 in tumor tissue was weaker upon lycopene compared to the placebo, but more intense upon lycopene + taxol and taxol + platin compared to lycopene + taxol + platin (mean ± SEM; n = 5; $P \leq 0.01$). Scale bars, 50 µm. F. Lycopene treatment significantly reduced MMP9 in serum and CA125 in ascites.

reduced *ITGA5* (P = 0.02) but increased *MMP9* (P = 0.001) levels compared to the placebo (**Figure 3D**). Comparing lycopene with platin treatment, levels of *ITGA5* (P < 0.0001), *ITGB1* (P = 0.0006), *MMP9* (P < 0.0001), *ILK* (P < 0.0001) and *FAK* (P < 0.0001) were significantly down-regulated upon lycopene treatment, further indicating that this tumor model might be less sensitive to platin. The expression of all tested genes was significantly reduced in the triple lycopene + taxol + platin combination treatment compared to taxol + platin, which was confirmed by quantitative image analysis (**Figure 3D**).

To determine the therapeutic impact of lycopene on the production of the integrin α5β1 heterodimer and MMP9, immunohistochemical analysis and ELISA were conducted. As seen for the lycopene prevention regimen, staining of integrin α5β1 in tumor tissue was weaker upon lycopene treatment compared to the placebo. Comparing the combination treatments, the triple lycopene + taxol + platin combination treatment had the most profound effect in reducing integrin α5β1 in tumor tissues compared to lycopene + taxol and taxol + platin (**Figure 3E**).

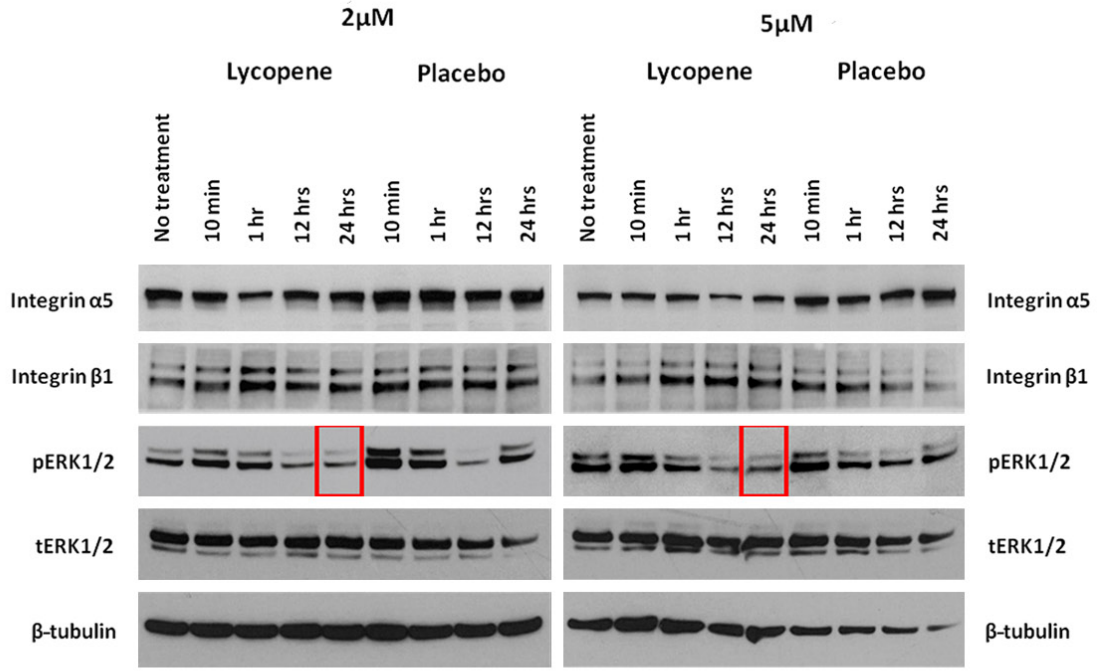
To validate the effects seen upon lycopene treatment, levels of human-derived MMP9 and CA125 were determined in serum and ascites. Lycopene treatment significantly reduced the level of MMP9 in serum but not in ascites compared to the placebo (**Figure 3F**, left panel). The CA125 level was significantly reduced in ascites upon lycopene treatment but not in serum compared to the placebo (**Figure 3F**, right panel). Overall, therapeutic administration of lycopene reduced tumor growth and cancer-mediating factors, with a further reduction when combined with clinically used chemotherapeutics.

Lycopene treatment altered the expression of integrin α5 and activation of MAPK

To elucidate the signaling mechanisms of lycopene-mediated effects, OV-MZ-6 cells were incubated with a physiological (2 µM) and supra-physiological (5 µM) dose of lycopene for up to 24 hrs [24]. Both lycopene concentrations reduced the protein expression of integrin α5 in OV-MZ-6 cells at all time points tested, but not integrin β1 (**Figure 4A**). However, since the data originate from two biological replicates, outcomes were not considered significant, although the relative integrin α5 expression ratio was considerably reduced upon lycopene treatment (**Figure 4B**). Treatment of OV-MZ-6 cells with 2 and 5 µM lycopene substantially reduced the phosphorylation of ERK1/2 after 24 hrs compared to the placebo (lycopene:placebo 0.23 ± 0.13 and 0.24 ± 0.12 respectively). Integrin β1 and total ERK1/2 levels did not change.

To validate the inhibitory effect of lycopene treatment on the phosphorylation of ERK1/2, immunohistochemical analysis of tumor and metastatic tissues was performed using the same antibodies and a human-specific antibody against vimentin as control staining [15]. Tumor tissues from animals treated with lycopene and the placebo showed no evidence of phosphorylation of ERK1/2 (**Figure 4C**). Strikingly, a strong phosphorylation of ERK1/2 was observed in the metastatic tissue from placebo-treated animals compared to the negative staining upon lycopene treatment (**Figure 4C**). The reduced phosphorylation of ERK1/2 is in agreement with the reduced intraperitoneal metastasis and proliferation

A



B

2μM

	Lycopene				Placebo			
	10 min	1 hr	12 hrs	24 hrs	10 min	1 hr	12 hrs	24 hrs
Integrin α5	0.97±0.13	0.67±0.19	0.80±0.0	0.75±0.11	1.0±0.24	1.0±0.22	1.0±0.13	1.0±0.36
pERK1/2	0.68±0.19	0.68±0.40	0.64±0.20	**0.23±0.13**	1.0±0.06	1.0±0.38	1.0±0.02	1.0±0.35

5μM

	Lycopene				Placebo			
	10 min	1 hr	12 hrs	24 hrs	10 min	1 hr	12 hrs	24 hrs
Integrin α5	0.58±0.08	0.62±0.12	0.60±0.0	0.36±0.08	1.0±0.46	1.0±0.45	1.0±0.49	1.0±0.39
pERK1/2	0.78±0.26	0.96±0.56	0.79±0.35	**0.24±0.12**	1.0±0.35	1.0±0.53	1.0±0.50	1.0±0.32

C

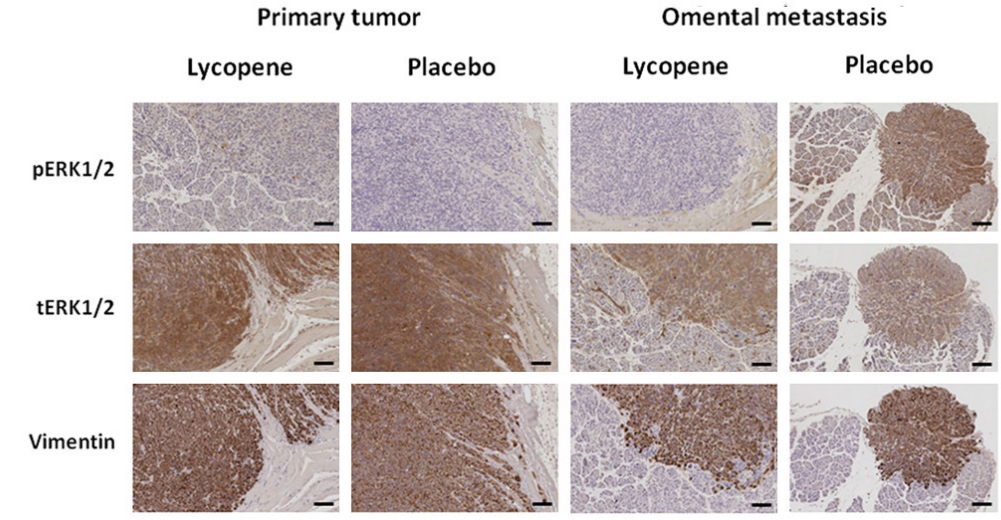


Figure 4. Effects of lycopene on signaling. A, B. Treatment of OV-MZ-6 cells with 2 and 5 μM lycopene reduced the expression of integrin α5. Phosphorylation of ERK1/2 was reduced at all time points with both concentrations, with a substantially reduction after 24 hrs of treatment (indicated in red). Integrin β1 and total ERK1/2 levels did not change. Values are normalized to no treatment and placebo controls. C. Immunohistochemical staining of pEKR1/2 in tumor tissue was negative and positive in metastatic tissue from placebo-treated animals. Total ERK1/2 and vimentin staining intensity were strong in both treatment regimen and tissue types. Scale bars, 100 μm.

seen in lycopene-treated animals. Total ERK1/2 and vimentin staining intensity was strong and unchanged in both treatment regimes and tissue types (**Figure 4C**). Overall, lycopene treatment decreased the protein expression of integrin α5 and inhibited ERK1/2 signaling, thus implying a role in integrin α5/MAPK signaling.

Discussion

Increasing evidence suggest that a tomato-based diet or the supplemental intake of lycopene has a number of health benefits and protective effects against heart disease and cancer [25]. Epidemiological studies indicate that natural products or nutritional supplements may suppress the growth and metastasis of different tumor entities [26]. Antioxidants are a major group of naturally-derived protective compounds that minimize cell damage generated by free radicals [27, 28]. The antioxidant properties of lycopene have been reported for the prevention and treatment of different tumor entities, especially in prostate cancer [10]. In ovarian cancer, the role of antioxidants is less studied.

Serous ovarian cancer, the most aggressive and prevalent type of ovarian cancer, originates from ovarian epithelial inclusion cysts and the fallopian tube epithelium [29]. This disease can be caused by malignant transformation of surface epithelial cells during ovulation [30, 31]. Ovulation induced in animals caused enhanced macrophage infiltration into the fallopian tube epithelium and DNA damage in surface epithelial cells [29]. The reduction of oxidative stress at surface epithelia might be an approach to prevent or treat serous ovarian cancer. To date, lycopene's potential preventive and therapeutic effects in ovarian cancer have not been studied [26]. To our knowledge, this is the first study demonstrating the impact of lycopene on ovarian cancer *in vivo*.

The preventive effect of lycopene on tumor growth and the therapeutic effect, as a single agent and in combination with clinically used chemotherapeutics, against established tumors were analyzed using a humanized approach. Clinically relevant disease models that recapitulate human tumor growth and metastasis are essential tools for advancing cancer research [32, 33]. Our group has shown that the humanized intraperitoneal animal model, used for this study, closely mimics human ovarian tumor growth and metastatic spread as seen in patients [14]. Lycopene prevention administered orally to NOD/SCID mice resulted in smaller tumors and significantly reduced intraperitoneal metastases compared to the placebo. Lycopene prevention decreased cell proliferation in primary tumors and metastases as demonstrated by weak Ki67 staining of both tissues. Tumor progression is promoted by integrin β1 [34, 35], and increased integrin expression in tumors positively correlates with cancer cell invasion [36]. In this study, gene expression of *ITGA5* and *ITGB1* was reduced upon lycopene prevention in both tumor and metastatic tissues. Correspondingly, a lower production of the integrin α5β1 heterodimer was observed in tumor tissue upon lycopene prevention compared to the placebo. A similar finding was reported by Bureyko et al [37], showing that lycopene-treated prostate cancer cells had a decreased expression of integrin α2β1.

Integrin signaling is mediated by interactions with the cytosolic ILK [38] and the cytoplasmic FAK [39]. ILK interacts with integrin β1 and β3 subunits, mediating interactions between cells and the extracellular matrix (ECM) to regulate signaling pathways involved in cancer cell growth, survival and invasion [17, 40]. ILK expression is up-regulated in ovarian cancer and positively correlates with tumor progression [17, 41]. ILK-deficient ovarian cancer cells have reduced tumorigenic potential *in vivo* [17]. FAK signaling is activated by integrin β1, β3 and β5 subunits, promoting cancer cell survival and invasion [19, 42]. FAK overexpression in ovarian cancer is associated with resistance to chemotherapeutics [39, 43]. In this study, gene expression of *ILK* and *FAK* were significantly reduced in tumors and metastases upon lycopene prevention.

EMT is a critical process that drives cancer development and progression [23]. In this study, we demonstrated that intraperitoneal metastatic tumors isolated from animals receiving lycopene prevention had significantly lower mRNA levels of all key EMT markers. These EMT genes are implicated in metastases, cancer cell invasion and disease progression [44]. These findings suggest that the preventative lycopene regimen reduced the

metastatic load of ovarian cancer in NOD/SCID mice by down-regulation of integrin α5β1, integrin-interacting factors and EMT genes to suppress the aggressive stage of this disease and distant metastases.

Besides EMT, MMPs are strongly associated with cancer cell invasion and metastasis by degrading ECM proteins and the basement membrane [45, 46]. MMP9 is up-regulated in ovarian cancer and leads to metastasis and poor prognosis [20], while down-regulation of MMP9 inhibits metastatic spread [47]. Tang et al [48] reported a reduction of MMP9 levels in serum of colon cancer-bearing mice after receiving a combination of lycopene and fish oil. In this study, the preventive administration of lycopene reduced the gene expression of *MMP9* in metastatic tissue and protein level in serum and ascites derived from ovarian cancer-bearing mice, without reaching significance. In addition, lycopene prevention significantly decreased the level of CA125 in serum and ascites. These findings further suggest that the preventive intake of lycopene decreased ovarian tumor burden and diminished metastatic spread.

We also investigated lycopene's potential as ovarian cancer treatment as a single agent and in combination with the clinically used chemotherapeutics taxol and platin to validate possible synergistic effects of lycopene with these cytotoxic drugs. Lycopene treatment was superior to the placebo in reducing the tumor burden. Interestingly, the lycopene + taxol combination treatment was as effective as the taxol + platin combination in reducing both the tumor and metastatic loads. Comparing lycopene and platin in a single agent regimen, lycopene reduced the metastatic load in ovarian cancer-bearing mice even further than platin. Thus, lycopene might be useful to reduce the development of intraperitoneal metastases that are less sensitive to platin.

Enhanced anti-tumor effects of cytotoxic drugs, such as docetaxel, in combination with lycopene have also been described by Tang et al [49] to treat prostate cancer-bearing mice. Similar to this study, integrin α5β1 was reduced at both gene and protein levels upon lycopene treatment. Compared to platin treatment, lycopene reduced the expression of *ITGA5*, *ITGB1*, *MMP9*, *FAK* and *ILK*. The addition of lycopene to the taxol + platin combination treatment had the most profound effect on all genes investigated. High levels of integrin β1 in ovarian cancer correlate with advanced stage and poor survival [50]. In this study, the triple lycopene + taxol + platin combination treatment reduced the production of the integrin α5β1 heterodimer even further than the taxol + platin and lycopene + taxol combinations. These findings indicate that lycopene synergistically enhanced anti-tumorigenic effects of chemotherapeutics and potentially minimises side effects of chemotherapy.

The expression of Ki67 in tumor tissues was lowered upon lycopene treatment compared to the placebo. The impact of lycopene on cancer cell proliferation has been reported by others [51-54]. Lycopene treatment with physiological and supra-physiological concentrations reduced the protein expression of integrin α5 and inhibited MAPK signaling. The integrin α5/MAPK pathway regulates different cell functions, including cell proliferation and survival [55, 56]. Not only lycopene, but also other naturally-derived compounds, such as dietary fats or cruciferous vegetables, which are part of the human diet, can influence MAPK signaling in different cancer entities, such as colon and rectal cancer [56]. El-Senduny et al [55] showed that cucurbitacin B in combination with cisplatin depleted the phosphorylation of ERK1/2, which sensitized ovarian cancer cells to cisplatin [55]. Our study was performed with ovarian cancer OV-MZ-6 cells, representative of serous ovarian cancer (type II). However, this humanized intraperitoneal animal model is a tool to further expand research into type I ovarian cancers.

Conclusions

Lycopene, administered orally to NOD/SCID mice in a preventive manner significantly reduced the intraperitoneal metastatic load and given as therapeutic significantly reduced the tumor load of ovarian cancer-bearing mice. Strikingly, lycopene synergistically enhanced the response of ovarian cancer cells to the clinically used chemotherapeutics paclitaxel and carboplatin. These anti-tumorigenic effects were mediated by a down-regulation of *ITGA5*, *ITGB1*, *MMP9*, *FAK*, *ILK* and EMT markers, all

factors involved in disease progression. Both preventive and therapeutic lycopene regimen decreased the expression of CA125, the clinically used ovarian tumor biomarker. Lycopene treatment of ovarian cancer OV-MZ-6 cells reduced the protein expression of integrin α5 and activation of MAPK signaling. To date, most studies on lycopene have been undertaken in prostate and breast cancer models [10]. This study demonstrates, for the first time, that lycopene exerts its antioxidative effects in a bioengineered disease model of ovarian cancer. These findings give reason to further expand lycopene research into the prevention and therapy of ovarian cancer.

Acknowledgements

The work presented by the authors was supported by the Australian Research Council (Australian Research Centre in Additive Biomanufacturing, D.W.H.; Discovery project grant to D.W.H., J.A.C. and D.L.), National Health and Medical Research Council of Australia (Principal Research Fellowship awarded to J.A.C.), Movember Foundation and the Prostate Cancer Foundation of Australia through a Movember Revolutionary Team Award (J.A.C. and D.W.H.), Queensland University of Technology (N.P.H. and D.L.), German Research Foundation (DFG; WA 3606/1-1 to F.W., HO 5068/1-1 to B.M.H.) and by a mobility grant (Personalized Medicine) from the German Academic Exchange Service (DAAD; M.L., J.A.C., D.W.H. and D.L.).

Disclosure of conflict of interest

None.

Authors' contribution

N.P.H., A.S., F.W., M.L. and B.M.H. conducted the research. S.C. provided the lycopene compound. J.A.C., D.W.H. and D.L. designed the research. N.P.H. and D.L. wrote the manuscript. All authors read and approved the final manuscript.

Abbreviations

ECL, Enhanced chemiluminescence; BLI, bioluminescence imaging; NuMA, nuclear mitotic apparatus protein 1; EMT, epithelial to mesenchymal transition; ECM, extracellular matrix.

Address correspondence to: Daniela Loessner, Institute of Health and Biomedical Innovation, Queensland University of Technology, 60 Musk Avenue, Kelvin Grove, QLD 4059, Brisbane, Australia. Tel: +61-7-3138-6441; Fax: +61-7-3138-6030; E-mail: daniela.lossner@qut.edu.au

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.

[2] Lengyel E. Ovarian cancer development and metastasis. Am J Pathol 2010; 177: 1053-64.

[3] Chandran U, Bandera EV, Williams-King MG, Paddock LE, Rodriguez-Rodriguez L, Lu SE, Faulkner S, Pulick K, Olson SH. Healthy eating index and ovarian cancer risk. Cancer Causes Control 2011; 22: 563-71.

[4] Gifkins D, Olson SH, Paddock L, King M, Demissie K, Lu SE, Kong AN, Rodriguez-Rodriguez L, Bandera EV. Total and individual antioxidant intake and risk of epithelial ovarian cancer. BMC Cancer 2012; 12: 211.

[5] van der Wijst MG, Brown R, Rots MG. Rots, Nrf2, the master redox switch: the Achilles' heel of ovarian cancer? Biochim Biophys Acta 2014; 1846: 494-509.

[6] Murdoch WJ, Martinchick JF. Oxidative damage to DNA of ovarian surface epithelial cells affected by ovulation: carcinogenic implication and chemoprevention. Exp Biol Med (Maywood) 2004; 229: 546-52.

[7] Rieck G, Fiander A. The effect of lifestyle factors on gynaecological cancer. Best Pract Res Clin Obstet Gynaecol 2006; 20: 227-51.

[8] Helzlsouer KJ, Alberg AJ, Norkus EP, Morris JS, Hoffman SC, Comstock GW. Prospective study of serum micronutrients and ovarian cancer. J Natl Cancer Inst 1996; 88: 32-7.

[9] Chan JM, Elkin EP, Silva SJ, Broering JM, Latini DM, Carroll PR. Total and specific complementary and alternative medicine use in a large cohort of men with prostate cancer. Urology 2005; 66: 1223-8.

[10] Holzapfel NP, Holzapfel BM, Champ S, Feldthusen J, Clements J, Hutmacher DW. The potential role of lycopene for the prevention and therapy of prostate cancer: from molecular mechanisms to clinical evidence. Int J Mol Sci 2013; 14: 14620-46.

[11] Peters U, Leitzmann MF, Chatterjee N, Wang Y, Albanes D, Gelmann EP, Friesen MD, Riboli E, Hayes RB. Serum lycopene, other carotenoids, and prostate cancer risk: a nested case-control study in the prostate, lung, colorectal, and ovarian cancer screening trial. Cancer Epidemiol Biomarkers Prev 2007; 16: 962-8.

[12] Li X. and Xu J. Meta-analysis of the association between dietary lycopene intake and ovarian

cancer risk in postmenopausal women. Sci Rep 2014; 4: 4885.
[13] Möbus V, Gerharz CD, Press U, Moll R, Beck T, Mellin W, Pollow K, Knapstein PG, Kreienberg R. Morphological, immunohistochemical, and biochemical characterization of 6 newly established human ovarian-carcinoma cell-lines. Int J Cancer 1992. 52: 76-84.
[14] Loessner D, Rizzi SC, Stok KS, Fuehrmann T, Hollier B, Magdolen V, Hutmacher DW, Clements JA. A bioengineered 3D ovarian cancer model for the assessment of peptidase-mediated enhancement of spheroid growth and intraperitoneal spread. Biomaterials 2013; 34: 7389-400.
[15] Loessner D, Holzapfel BM, Clements JA. Engineered microenvironments provide new insights into ovarian and prostate cancer progression and drug responses. Adv Drug Deliv Rev 2014; 79-80: 193-213.
[16] Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000; 182: 311-22.
[17] Li Q, Li C, Zhang YY, Chen W, Lv JL, Sun J, You QS. Silencing of integrin-linked kinase suppresses in vivo tumorigenesis of human ovarian carcinoma cells. Mol Med Rep 2013; 7: 1050-4.
[18] Loessner D, Stok KS, Lutolf MP, Hutmacher DW, Clements JA, Rizzi SC. Bioengineered 3D platform to explore cell-ECM interactions and drug resistance of epithelial ovarian cancer cells. Biomaterials 2010; 31: 8494-506.
[19] Tancioni I, Uryu S, Sulzmaier FJ, Shah NR, Lawson C, Miller NL, Jean C, Chen XL, Ward KK, Schlaepfer DD. FAK Inhibition disrupts a beta5 integrin signaling axis controlling anchorage-independent ovarian carcinoma growth. Mol Cancer Ther 2014; 13: 2050-61.
[20] Guo F, Tian J, Cui M, Fang M, Yang L. Downregulation of matrix metalloproteinase 9 by small interfering RNA inhibits the tumor growth of ovarian epithelial carcinoma in vitro and in vivo. Mol Med Rep 2015; 12: 753-9.
[21] Mitra AK, Sawada K, Tiwari P, Mui K, Gwin K, Lengyel E. Ligand-independent activation of c-Met by fibronectin and alpha(5)beta(1)-integrin regulates ovarian cancer invasion and metastasis. Oncogene 2011; 30: 1566-76.
[22] Bast RC Jr, Badgwell D, Lu Z, Marquez R, Rosen D, Liu J, Baggerly KA, Atkinson EN, Skates S, Zhang Z, Lokshin A, Menon U, Jacobs I, Lu K. New tumor markers: CA125 and beyond. Int J Gynecol Cancer 2005; 15 Suppl 3: 274-81.
[23] De Craene B, Berx G. Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer 2013; 13: 97-110.
[24] Allen CM, Schwartz SJ, Craft NE, Giovannucci EL, De Groff VL, Clinton SK. Changes in plasma and oral mucosal lycopene isomer concentrations in healthy adults consuming standard servings of processed tomato products. Nutr Cancer 2003; 47: 48-56.
[25] Thies F, Mills LM, Moir S, Masson LF. Cardiovascular benefits of lycopene: fantasy or reality? Proc Nutr Soc 2016; 1-8.
[26] Feitelson MA, Arzumanyan A, Kulathinal RJ, Blain SW, Holcombe RF, Mahajna J, Marino M, Martinez-Chantar ML, Nawroth R, Sanchez-Garcia I, Sharma D, Saxena NK, Singh N, Vlachostergios PJ, Guo S, Honoki K, Fujii H, Georgakilas AG, Bilsland, Amedei A, Niccolai E, Amin A, Ashraf SS, Boosani CS, Guha G, Ciriolo MR, Aquilano K, Chen S, Mohammed SI, Azmi AS, Bhakta D, Halicka D, Keith WN, Nowsheen S. Sustained proliferation in cancer: mechanisms and novel therapeutic targets. Semin Cancer Biol 2015; 35 Suppl: S25-54.
[27] Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord JM, Harman D. Oxygen radicals and human disease. Ann Intern Med 1987; 107: 526-45.
[28] Diplock AT. Antioxidant nutrients and disease prevention: an overview. Am J Clin Nutr 1991; 53 Suppl: 189S-193S.
[29] King SM, Hilliard TS, Wu LY, Jaffe RC, Fazleabas AT, Burdette JE. The impact of ovulation on fallopian tube epithelial cells: evaluating three hypotheses connecting ovulation and serous ovarian cancer. Endocr Relat Cancer 2011; 18: 627-42.
[30] Murdoch WJ, McDonnel AC. Roles of the ovarian surface epithelium in ovulation and carcinogenesis. Reproduction 2002; 123: 743-50.
[31] Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. J Natl Cancer Inst 1999; 91: 1459-67.
[32] Holzapfel BM, Wagner F, Thibaudeau L, Levesque JP, Hutmacher DW. Concise review: Humanized models of tumor immunology in the 21st century: convergence of cancer research and tissue engineering. Stem Cells 2015; 33: 1696-704.
[33] Hutmacher DW, Holzapfel BM, De-Juan-Pardo EM, Pereira BA, Ellem SJ, Loessner D, Risbridger GP. Convergence of regenerative medicine and synthetic biology to develop standardized and validated models of human diseases with clinical relevance. Curr Opin Biotechnol 2015; 35: 127-132.
[34] Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat Rev Cancer 2003; 3: 362-74.
[35] Guo W, Giancotti FG. Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 2004; 5: 816-26.
[36] Lee LK, KYF. An appraisal of the therapeutic value of lycopene for the chemoprevention of prostate cancer: a nutrigenomic approach. Food Res Int 2013; 54: 1217-1228.

[37] Bureyko T, Hurdle H, Metcalfe JB, Clandinin MT, Mazurak VC. Reduced growth and integrin expression of prostate cells cultured with lycopene, vitamin E and fish oil in vitro. Br J Nutr 2009; 101: 990-7.
[38] Hannigan GE, Leung-Hagesteijn C, Fitz-Gibbon L, Coppolino MG, Radeva G, Filmus J, Bell JC, Dedhar S. Regulation of cell adhesion and anchorage-dependent growth by a new beta 1-integrin-linked protein kinase. Nature 1996; 379: 91-6.
[39] Yoon H, Choi YL, Song JY, Do I, Kang SY, Ko YH, Song S, Kim BG. Targeted inhibition of FAK, PYK2 and BCL-XL synergistically enhances apoptosis in ovarian clear cell carcinoma cell lines. PLoS One 2014; 9: e88587.
[40] McDonald PC, Fielding AB, Dedhar S. Integrin-linked kinase--essential roles in physiology and cancer biology. J Cell Sci 2008; 121: 3121-32.
[41] Choi YP, Kim BG, Gao MQ, Kang S, Cho NH. Targeting ILK and beta4 integrin abrogates the invasive potential of ovarian cancer. Biochem Biophys Res Commun 2012; 427: 642-8.
[42] Yousif NG. Fibronectin promotes migration and invasion of ovarian cancer cells through up-regulation of FAK-PI3K/Akt pathway. Cell Biol Int 2014; 38: 85-91.
[43] McLean GW, Carragher NO, Avizienyte E, Evans J, Brunton VG, Frame MC. The role of focal-adhesion kinase in cancer-a new therapeutic opportunity. Nat Rev Cancer 2005; 5: 505-15.
[44] Gardi NL, Deshpande TU, Kamble SC, Budhe SR, Bapat SA. Discrete molecular classes of ovarian cancer suggestive of unique mechanisms of transformation and metastases. Clin Cancer Res 2014; 20: 87-99.
[45] Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000; 18: 1135-49.
[46] Palozza P, Simone RE, Catalano A, Mele MC. Tomato lycopene and lung cancer prevention: from experimental to human studies. Cancers 2011; 3: 2333-57.
[47] He J, Xu Q, Wang M, Li C, Qian X, Shi Z, Liu LZ, Jiang BH. Oral administration of apigenin inhibits metastasis through AKT/P70S6K1/MMP-9 pathway in orthotopic ovarian tumor model. Int J Mol Sci 2012; 13: 7271-82.
[48] Tang FY, Pai MH, Kuo YH, Wang XD. Concomitant consumption of lycopene and fish oil inhibits tumor growth and progression in a mouse xenograft model of colon cancer. Mol Nutr Food Res 2012; 56: 1520-31.
[49] Tang Y, Parmakhtiar B, Simoneau AR, Xie J, Fruehauf J, Lilly M, Zi X. Lycopene enhances docetaxel's effect in castration-resistant prostate cancer associated with insulin-like growth factor I receptor levels. Neoplasia 2011; 13: 108-19.
[50] Lau MT, So WK, Leung PC. Integrin beta1 mediates epithelial growth factor-induced invasion in human ovarian cancer cells. Cancer Lett 2012; 320: 198-204.
[51] Hwang ES, Bowen PE. Effects of tomato paste extracts on cell proliferation, cell-cycle arrest and apoptosis in LNCaP human prostate cancer cells. Biofactors 2005; 23: 75-84.
[52] Mueller E, Smith M, Sarraf P, Kroll T, Aiyer A, Kaufman DS, Oh W, Demetri G, Figg WD, Zhou XP, Eng C, Spiegelman BM, Kantoff PW. Effects of ligand activation of peroxisome proliferator-activated receptor gamma in human prostate cancer. Proc Natl Acad Sci U S A 2000; 97: 10990-5.
[53] Teodoro AJ, Oliveira FL, Martins NB, Maia Gde A, Martucci RB, Borojevic R. Effect of lycopene on cell viability and cell cycle progression in human cancer cell lines. Cancer Cell Int 2012; 12: 36.
[54] Uppala PT, Dissmore T, Lau BH, Andacht T, Rajaram S. Selective inhibition of cell proliferation by lycopene in MCF-7 breast cancer cells in vitro: a proteomic analysis. Phytother Res 2013; 27: 595-601.
[55] El-Senduny FF, Badria FA, El-Waseef AM, Chauhan SC, Halaweish F. Approach for chemosensitization of cisplatin-resistant ovarian cancer by cucurbitacin B. Tumour Biol 2015; 37: 685-98.
[56] Slattery ML, Lundgreen A, Wolff RK. Dietary influence on MAPK-signaling pathways and risk of colon and rectal cancer. Nutr Cancer 2013; 65: 729-38.