Characterizing the Efficacy of Fermented Wheat Germ Extract Against Ovarian Cancer and Defining the Genomic Basis of Its Activity

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Objective: Most women with advanced-stage epithelial ovarian cancer (OVCA) ultimately develop chemoresistant recurrent disease. Therefore, a great need to develop new, more active, and less toxic agents and/or to optimize the efficacy of existing agents exists.

Methods: In this study, we investigated the activity of Avemar, a natural, nontoxic, fermented wheat germ extract (FWGE), against a range of OVCA cell lines, both alone and in combination with cisplatin chemotherapy and delineated the molecular signaling pathways that underlie FWGE activity at a genome-wide level.

Results: We found that FWGE exhibited significant antiproliferative effects against 12 human OVCA cell lines and potentiated cisplatin-induced apoptosis. Pearson correlation of FWGE sensitivity and gene expression data identified 2142 genes (false discovery rate < 0.2) representing 27 biologic pathways ($P < 0.05$) to be significantly associated with FWGE sensitivity. A parallel analysis of genomic data for 59 human cancer cell lines matched to chemosensitivity data for 2,6-dimethoxy-*p*-benzoquinone, a proposed active component of FWGE, identified representation of 13 pathways common to both FWGE and 2,6-dimethoxy-*p*-benzoquinone sensitivity.

Conclusions: Our findings confirm the value of FWGE as a natural product with anticancer properties that may also enhance the activity of existing therapeutic agents. Furthermore, our findings provide substantial insights into the molecular basis of FWGE's effect on human cancer cells.

Research Highlights:

- Fermented wheat germ extract has significant antiproliferative effects on OVCA cell lines and may enhance the effect of cisplatin-induced cell death.
- Genome-wide expression data reveal that FWGE sensitivity in ovarian cancer cells was associated with 2142 genes, representing 27 biologic pathways.

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• The known safety and tolerability of FWGE supports the clinical evaluation of this natural product in patients with ovarian cancer.

Key Words: Fermented wheat germ extract, Avemar, Ovarian cancer, Apoptosis, Cisplatin

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In 2012, more than 22,000 women in the United States will be diagnosed with epithelial ovarian cancer (OVCA) and 15,500 women will die of the disease.[1] Approximately 75% of patients with OVCA are diagnosed at an advanced stage (III/IV), with disseminated intraperitoneal metastases.[2] Initially, patients with advanced-stage OVCA are treated with primary cytoreductive surgery followed by chemotherapy with a platinum/taxane–based chemotherapy regimen.

Although approximately 70% of women will experience a complete clinical response to initial therapy, the majority will develop platinum-resistant, progressive, or recurrent disease. Patients with platinum-resistant OVCAs often demonstrate cross-resistance to most other chemotherapeutic agents; these women have a poor prognosis and are subject to empirically driven treatment with multiple chemotherapeutics; response rates to these are generally less than 20%. During such treatment, patients experience significant toxicities, compromise to bone marrow reserves, detriment to quality of life, and delay in the initiation of active agents. The development of chemoresistance is a critical determinant of survival for women with OVCA, and it is generally accepted that patients lose their battle with the disease when chemoresistance develops. The incidence and lethality of the epithelial OVCA underscore the need to improve our therapeutic approaches while decreasing the toxicity associated with treatment.

Up to 89% of patients with cancer or other chronic conditions use integrative, complementary, or alternative therapies, often including herbal or natural products.[3,4] Most of these products have not been subjected to comprehensive study for efficacy or potential negative interactions with chemotherapy. Natural, nontoxic regimens that enhance standard-of-care therapy and/or prolong progression-free survival, while maintaining quality of life, are highly desirable in treatment of patients with cancer.

Avemar is a fermented wheat germ extract (FWGE) that was developed by Dr. Mate Hidvegi in Hungary in the early 1990s.[5] It is produced by extraction of wheat germ, fermentation of the extract, separation of the fermentation liquid, concentration, and drying. The chemical composition of FWGE is a mixture of molecules, including 2-methoxy-*p*-benzoquinone and 2,6-dimethoxy-*p*-benzoquinone, which may contribute to its biologic properties.[6] Fermented wheat germ extract has been evaluated in vitro and shown to induce apoptosis in many cancer cell types, including leukemia, melanoma, breast, colon, testicular, head and neck, cervical, ovarian, gastric, thyroid, and brain carcinomas.[7–9] Observations from human clinical trials suggest beneficial effects of FWGE on disease progression and survival in patients with melanoma[10] and colorectal cancer.[11]

Despite reported data that FWGE induces apoptosis and has significant antitumor activity in many cancer cell types, FWGE has not been fully characterized for activity against OVCA. Moreover, the effects of FWGE on OVCA sensitivity to chemotherapy remain to be determined. Mueller et al[7] demonstrated FWGE in vitro activity against a single OVCA cell line, A2780, but did not investigate how FWGE interacts with cytotoxic agents against OVCA cells.

The biologic basis to FWGE activity against OVCA and the proportion of women with the disease who may benefit are both unclear. We therefore sought to investigate the activity of FWGE against a range of OVCA cell lines, both alone and in combination with cisplatin chemotherapy. Furthermore, we also aimed to delineate the molecular signaling pathways that underlie FWGE activity at a genome-wide level.

MATERIALS AND METHODS

Overview

Twelve human OVCA cell lines were subjected to treatment with FWGE with or without (+/−) the addition of cisplatin and parallel microarray expression analysis. Sensitivity to FWGE +/− cisplatin was quantified by MTS proliferation assays. Correlation analysis was used to identify genes associated with FWGE +/− cisplatin sensitivity. These genes were subjected to pathway analysis in an effort to characterize the biologic basis to FWGE effect. The study was performed with approval from the University of South Florida Institutional Review Board.

Cell Line Cultures

Ovarian cancer cell lines were either obtained from the American Type Culture Collection, Manassas, VA (SKOV3); the European Collection of Cell Cultures, Salisbury, England (A2780CP, A2780S); and Kyoto University, Kyoto, Japan (CHI, CHIcisR, M41, M41cisR, Tyknu, and TyknuCisR); or were kind gifts from Dr Patricia Kruk, Department of Pathology, College of Medicine, University of South Florida, Tampa, FL, and Susan Murphy, PhD, Department of OB/GYN, Division of GYN Oncology, Duke University, Durham, NC

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(OVCAR8, CAOV2, HeyA8). Cell lines were maintained in RPMI-1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Fisher Scientific, Pittsburgh, PA), 1% sodium pyruvate, 1% penicillin/streptomycin, and 1% non-essential amino acids (HyClone, Hudson, NH). *Mycoplasma* testing was performed every 6 months following the manufacturer's protocol (Lonza, Rockland, ME).

Drugs and Reagents

Fermented wheat germ extract was a gift from the manufacturer (American BioSciences, Inc, Blauvelt, NY). Fermented wheat germ extract was stored as dried powder at 4°C. Fermented wheat germ extract was solubilized immediately before each application in phosphate-buffered saline at a concentration of 40 mg/mL and then passed through a 0.22-μm polyethersulfone filter to remove any insoluble materials. Cisplatin was purchased from Sigma Aldrich, Inc (St. Louis, MO). RPMI-1640 was obtained from Invitrogen, Inc (Grand Island, NY), penicillin/streptomycin solution was obtained from Mediatech, Inc (Herndon, VA), and fetal bovine serum was purchased from Thermo Fisher Scientific (Waltham, MA).

Cell Viability Assays

The MTS assay was used to assess viability of the OVCA cell lines. For the assays, 3 to 5 $\times 10^4$ cells in 100 μL were plated to each well of a 96-well plate and allowed to adhere overnight at 37°C and 5% CO_2. The following day, cells were incubated with increasing concentrations, from 100 to 1000 μg/mL, of FWGE alone or with serial dilutions of cisplatin, starting at 100 μM, for 72 hours. Cell viability was analyzed using the CellTiter96 MTS assay kit (Promega, Madison, WI). Three replicate wells were used for each drug concentration, and an additional 3 control wells received a diluent control without drug. After drug incubation, the optical density of each well was read at 490 nm using a SpectraMax 190 microplate reader (Molecular Devices, Inc, Sunnyvale, CA). Percent cell survival was expressed as (control − treated) / (control − blank) × 100. The concentration at which cell viability was decreased by 50% was used to define the IC_{50}. All experiments were performed 3 times or the minimum number of times to ensure reproducibility and accuracy of the results.

RNA Extraction and Microarray Expression Analysis

RNA from the 12 OVCA cell lines was extracted using RNeasy kit following manufacturer's recommendations (Qiagen, Valencia, CA). The quality of the RNA was measured using an Agilent 2100 Bioanalzyer. The targets for Affymetrix DNA microarray analysis were prepared according to the manufacturer's instructions, and targets were hybridized to customized Human Affymetrix HuRSTA gene chips (HuRSTA-2a520709), which included 60,607 probe sets and representation of 19,308 genes (Gene Expression Omnibus accession number GSE34615).

Statistical Methods

The IC_{50} values for FWGE ± cisplatin were computed using sigmoidal dose-response algorithm implemented in GraphPad (GraphPad Software, Inc, San Diego, CA). Expression data from the 12 OVCA cell lines were subjected to background correction and normalization using the Robust Multichip Average algorithm in the Affymetrix Expression Console (http://media.affymetrix.com/support/downloads/manuals/expression_console_userguide.pdf). Pearson correlation test was performed on individual gene expression and IC_{50} values using Significance Analysis of Microarray software. Probe sets with a false discovery rate (FDR) less than 0.2 were considered to have significant correlations with IC_{50} values and were uploaded to MetaCore GeneGo for pathway analysis (http://www.genego.com/metacore.php). Pathways with FDR < 0.05 were considered significantly expressed, based on the GeneGo/MetaCore statistical test for significance.

Pathways Associated With Activity of FWGE Active Agents

In an effort to further explore the biologic basis to FWGE activity, we performed an in silico analysis of publicly available genome-wide expression and chemosensitivity data for 2,6-dimethoxy-*p*-benzoquinone, a molecule postulated to contribute to FWGE's activity[6] and 59 human cancer cell lines. In brief, chemosensitivity (GI50) data for 2,6-dimethoxy-*p*-benzoquinone and gene expression data for the NCI-60 panel of cell lines (which included 6 leukemia, 9 melanoma, 9 non–small cell lung, 7 colon, 6 central nervous system, 7 ovarian, 8 renal, 2 prostate, and 6 breast cancer cell lines) were obtained from NCI Web sites (http://discover.nci.nih.gov/cellminer/loadDownload.do and http://dtp.nci.nih.gov/dtpstandard/cancerscreeningdata/index.jsp). Pearson correlation was performed on 2,6-dimethoxy-*p*-benzoquinone GI50 values and gene expression data for 59/60 cancer cell lines (no gene expression data are available for 1 prostate cell line). Genes that demonstrated expression values that correlated with 2,6-dimethoxy-*p*-benzoquinone GI50 (FDR < 0.2) were subjected to GeneGo/MetaCore pathway analyses as described previously. Biologic pathways identified (FDR, $P < 0.05$) to be represented by genes associated with 2,6-dimethoxy-*p*-benzoquinone sensitivity in the NCI-60 cell lines were compared with pathways represented by genes associated with FWGE sensitivity, identified in a similar analysis of the 12 OVCA cell lines described previously.

RESULTS

Effect of FWGE ± Cisplatin on OVCA Cell Viability

The cytotoxic effects of continuous exposure to FWGE were assessed for 12 OVCA cell lines at 72 hours using the MTS assay. The IC_{50} values were calculated using a sigmoidal dose-response algorithm and ranked in order of FWGE sensitivity (Fig. 1). The median IC_{50} was 244.7 μg/mL. The OVCA cell lines demonstrating extreme sensitivity to FWGE included

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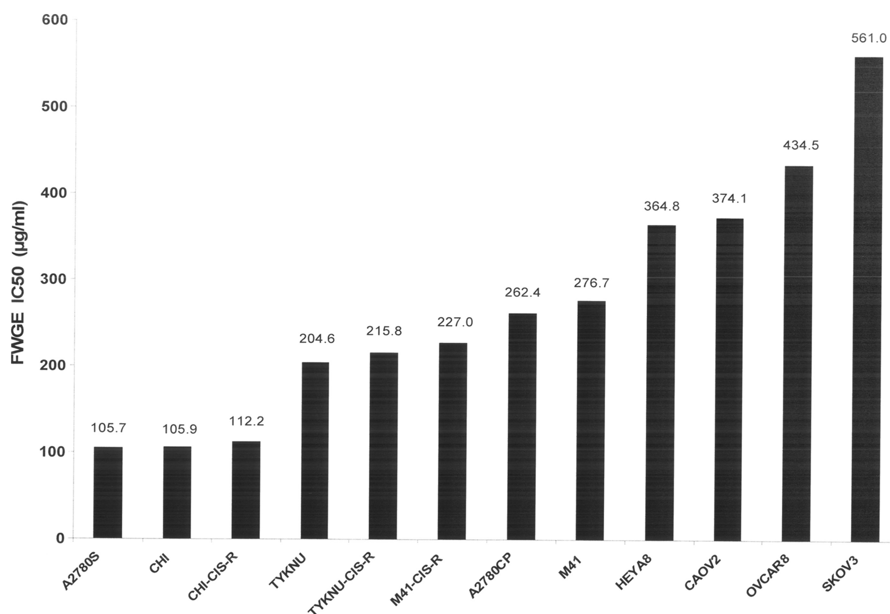

FIGURE 1. Fermented wheat germ extract inhibits OVCA cell line proliferation. The concentration of FWGE required to decrease OVCA cell viability by 50% (IC_{50}) was determined using the CellTiter96 MTS assay kit. Experiments were performed 3 times, or the minimum number of times to ensure reproducibility. Cell lines are ranked from most sensitive to most resistant.

SKOV3, which was most resistant (FWGE IC_{50} = 561 μg/mL) and A2780S, which was most sensitive (FWGE IC_{50} = 105.7 μg/mL).

In an effort to explore the effects of FWGE on OVCA cisplatin sensitivity, a fixed dose of FWGE was selected (approximating the FWGE IC_{30}), and the effect on cisplatin IC_{50} was evaluated in the 12 OVCA cell lines. All 12 cell lines demonstrated a decrease in cisplatin IC_{50} in the presence of FWGE. When evaluated together, the mean cisplatin IC_{50} (n = 12) was lower in the presence, versus in the absence, of FWGE (mean IC_{50} reduction = 1.11 μM, $P < 0.05$). Nine (75%) of 12 cell lines demonstrated a statistically significant decrease in cisplatin IC_{50} in the presence of FWGE versus cisplatin alone (A2780S, $P = 0.03$; CHI, $P = 0.001$; CHI-Cis-R, $P < 0.0001$; TYKNU, $P = 0.004$; TYKNU-Cis-R, $P = 0.01$; A2780CP, $P < 0.0001$; M41, $P = 0.03$; HEYA8, $P < 0.0001$; and SKOV3, $P = 0.02$). The FWGE-induced reduction in cisplatin IC_{50} did not reach statistical significance in 3 cell lines (CAOV2, $P = 0.3$; OVCAR8, $P = 0.2$; and M41-CIS-R, $P = 0.2$). The greatest change to cisplatin IC_{50} was observed in the platinum-resistant cell line, HEYA8 (6-fold; $P \leq 0.0001$). No antagonistic effects were observed (Table 1 and Fig. 2).

Genes and Signaling Pathways Associated With FWGE Sensitivity

Pearson correlation analysis of genome-wide expression data from the 12 OVCA cell lines and single-agent FWGE IC_{50} identified expression of 4033 probe sets, representing 2142 genes, to be associated with FWGE sensitivity (IC_{50}, FDR < 0.2) (Supplemental Digital Content 1, http://links.lww.com/IGC/A100). GeneGo/MetaCore pathway analysis of the probe sets and genes associated with FWGE sensitivity (FDR < 0.2) identified representation of 27 pathways ($P < 0.05$; Table 2), including cell cycle regulation of G_1/S transition, apoptosis and survival/granzyme A signaling, and cytoskeleton remodeling.

TABLE 1. Effect of FWGE on cisplatin IC_{50} on 12 OVCA cell lines

Ovarian Cell Line	IC_{50} FWGE, μg/mL	IC_{50} Cisplatin, μM	IC_{50} Cisplatin-FWGE, μM	*P*
A2780S	105.7	0.43	0.25	0.0273
CHI	105.9	0.21	0.14	0.0007
CHI-CIS-R	112.2	0.19	0.09	<0.0001
TYKNU	204.6	0.31	0.22	0.0045
TYKNU-CIS-R	215.8	1.40	0.68	0.0081
M41-CIS-R	227.0	5.16	4.39	0.2438
A2780CP	262.4	3.59	1.67	<0.0001
M41	276.7	2.09	1.20	0.0255
HEYA8	364.8	3.06	0.56	<0.0001
CAOV2	374.1	0.39	0.34	0.3387
OVCAR8	434.5	2.07	1.72	0.2271
SKOV3	561.0	1.21	0.92	0.0251

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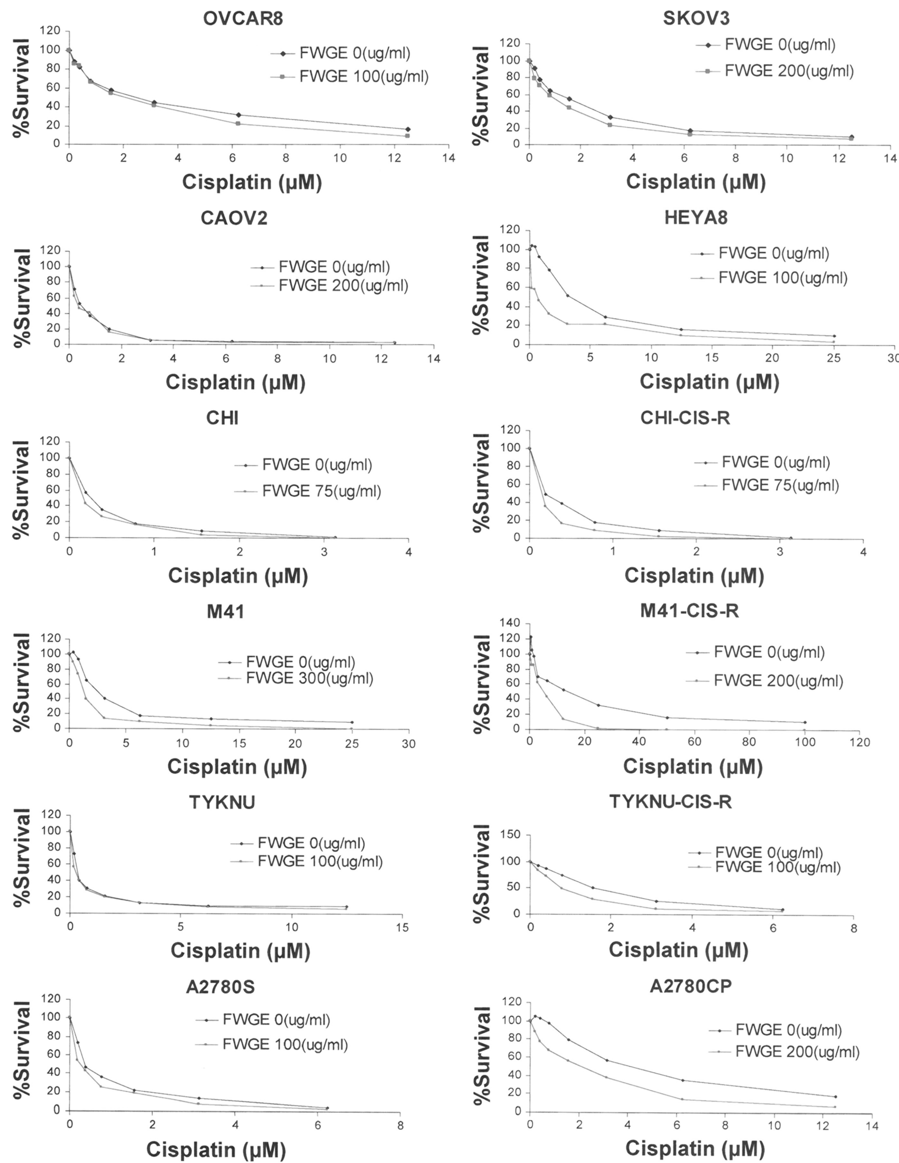

FIGURE 2. Fermented wheat germ extract potentiates growth arrest induced by cisplatin. The effects of FWGE on cisplatin-induced growth arrest at 72 hours was evaluated in OVCA cell lines using the CellTiter96 MTS assays. In an effort to isolate the effects of FWGE on cisplatin sensitivity, cells were incubated in the presence of increasing concentrations of cisplatin in the presence and absence of a fixed dose of FWGE at the approximate IC_{30} for each cell line. All experiments were performed a minimum of 3 times.

Genes and Molecular Signaling Pathways Associated With 2,6-Dimethoxy-*p*-benzoquinone Sensitivity

In a similar fashion, and in an effort to reconcile the biologic basis to FWGE activity with the biologic basis to one of its proposed active components, publicly available NCI-60 genomic and chemosensitivity data were analyzed for genes associated with 2,6-dimethoxy-*p*-benzoquinone sensitivity. This analysis identified 7251 probe sets representing 3839 genes (FDR < 0.2) (Supplemental Digital Content 2, http://links.lww.com/IGC/A101). GeneGo/MetaCore pathway analysis of genes/probe sets associated with 2,6-dimethoxy-*p*-benzoquinone sensitivity identified 267 pathways (FDR < 0.05) (Supplemental Digital Content 3, http://links.lww.com/IGC/A102).

Genes and Pathways Associated With Sensitivity to Both FWGE and 2,6-Dimethoxy-*p*-benzoquinone

Comparison of pathways associated with FWGE sensitivity and 2,6-dimethoxy-*p*-benzoquinone sensitivity identified 13 common pathways, including apoptosis and survival/granzyme A signaling, apoptosis and survival/granzyme B signaling, cell cycle/chromosome condensation in prometaphase, cell cycle/regulation of G_1/S transition, cell cycle/role of

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TABLE 2. FWGE pathway: gene cutoff, FDR < 0.2; pathway cutoff, FDR < 0.05

No.	Pathway Name	Pathway FDR	Pathway *P*	No. Objects Identified/ No. Objects in Pathway
1	Development_Hedgehog signaling	<0.05	$5.57e-09$	17/46
2	Signal transduction_Activin A signaling regulation	<0.06	$1.56e-08$	14/33
3	Cell cycle_Regulation of G_1/S transition (part 1)	<0.07	$6.88e-06$	12/38
4	Development_TGF-β receptor signaling	<0.08	$3.00e-05$	13/50
5	Regulation of metabolism_Bile acids regulation of glucose and lipid metabolism via FXR	<0.09	$3.16e-05$	11/37
6	Normal and pathological TGF-β–mediated regulation of cell proliferation	<0.10	$6.05e-05$	10/33
7	Cytoskeleton remodeling_TGF, WNT, and cytoskeletal remodeling	<0.11	$8.56e-05$	20/111
8	Apoptosis and survival_Granzyme A signaling	<0.12	$1.55e-04$	9/30
9	Development_WNT signaling pathway. Part 1. Degradation of β-catenin in the absence WNT signaling	<0.13	$2.03e-04$	7/19
10	Development_β-Adrenergic receptors signaling via cAMP	<0.14	$2.10e-04$	12/52
11	Cytoskeleton remodeling_Neurofilaments	<0.15	$2.19e-04$	8/26
12	Neurophysiological process_Receptor-mediated axon growth repulsion	<0.16	$2.23e-04$	11/45
13	Regulation of lipid metabolism_Regulation of lipid metabolism via LXR, NF-Y and SREBP	<0.17	$2.24e-04$	10/38
14	Apoptosis and survival_Granzyme B signaling	<0.18	$2.67e-04$	9/32
15	Development_BMP signaling	<0.19	$3.44e-04$	9/33
16	Cell cycle_Chromosome condensation in prometaphase	<0.20	$4.13e-04$	7/21
17	Regulation of lipid metabolism_Insulin regulation of fatty acid metabolism	<0.21	$4.40e-04$	16/89
18	Transcription_Role of heterochromatin protein 1 (HP1) family in transcriptional silencing	<0.22	$5.70e-04$	7/22
19	Development_Role of IL-8 in angiogenesis	<0.23	$6.14e-04$	12/58
20	Regulation of CFTR activity (norm and CF)	<0.24	$6.14e-04$	12/58
21	Development_Glucocorticoid receptor signaling	<0.25	$1.02e-03$	7/24
22	Cytoskeleton remodeling_Reverse signaling by ephrin B	<0.26	$1.10e-03$	8/31
23	Cell cycle_Role of Nek in cell cycle regulation	<0.27	$1.37e-03$	8/32
24	Regulation of lipid metabolism_Regulation of fatty acid synthase activity in hepatocytes	<0.28	$1.49e-03$	6/19
25	Development_Role of Activin A in cell differentiation and proliferation	<0.29	$1.56e-03$	9/40
26	Development_Melanocyte development and pigmentation	<0.30	$1.92e-03$	10/49
27	Cytoskeleton remodeling_Cytoskeleton remodeling	<0.31	$2.01e-03$	16/102

Nek in cell cycle regulation, cytoskeleton remodeling, cytoskeleton remodeling/reverse signaling by ephrin B, cytoskeleton remodeling/transforming growth factor (TGF), WNT and cytoskeletal remodeling, development/BMP signaling, development/melanocyte development and pigmentation, neurophysiological process/receptor–mediated axon growth repulsion, regulation of CFTR activity, and transcription/role of heterochromatin protein 1 family in transcriptional silencing (Table 3). That is, 13 (48%) of 27 pathways associated with FWGE sensitivity (FDR < 0.2) are also associated with sensitivity to 2,6-dimethoxy-*p*-benzoquinone.

DISCUSSION

In this study, we have shown that a natural, nontoxic FWGE, Avemar, exhibits antiproliferative and cytotoxic effects when applied on 12 different human OVCA cell lines. Fermented wheat germ extract resulted in a significant reduction

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TABLE 3. Common pathways between FWGE and 2,6-dimethoxy-*p*-benzoquinone

No.	Common Pathways
1	Apoptosis and survival_Granzyme A signaling
2	Apoptosis and survival_Granzyme B signaling
3	Cell cycle_Chromosome condensation in prometaphase
4	Cell cycle_Regulation of G_1/S transition (part 1)
5	Cell cycle_Role of Nek in cell cycle regulation
6	Cytoskeleton remodeling_Cytoskeleton remodeling
7	Cytoskeleton remodeling_Reverse signaling by ephrin B
8	Cytoskeleton remodeling_TGF, WNT, and cytoskeletal remodeling
9	Development_BMP signaling
10	Development_Melanocyte development and pigmentation
11	Neurophysiological process_Receptor-mediated axon growth repulsion
12	Regulation of CFTR activity (norm and CF)
13	Transcription_Role of heterochromatin protein 1 (HP1) family in transcriptional silencing

in OVCA survival after treating for 72 hours in all 12 cell lines. Although IC_{50} was not uniform among the OVCA cells treated with FWGE, the range between the least sensitive and most highly sensitive was relatively small at 455 μg/mL. The IC_{50} for the least sensitive OVCA cell line was lower than the estimated peak plasma concentration after oral intake of standard dose of 9 g/d FWGE (0.5–1 mg/mL),[8] suggesting that oral administration of FWGE in the form of Avemar may have therapeutic uses in women with OVCA. The observed differences in FWGE IC_{50} between various cell lines are likely a reflection of differences in biology between cells, either innate to their tumor of origin or acquired subsequently during passages. Such differences may be driven by DNA sequence variants (mutations or single-nucleotide polymorphisms in key genes that influence metabolism, cell and cell compartment pumps, apoptosis pathways, cell survival, etc), microRNA levels as previously described by our group,[12] messenger RNA expression levels and the molecular signaling pathways therein represented, or posttranslational events that influence apoptotic thresholds (eg, phosphorylation of the BAD protein[13]). We believe that our data provide some insights into the role of expression of molecular signaling pathways in determination of FWGE activity but also recognize that other parameters undoubtedly contribute to the process.

Fermented wheat germ extract resulted in the potentiation of the cisplatin-induced apoptosis in almost all OVCA cell lines. The IC_{50} of cisplatin was decreased significantly ($P < 0.05$) in 9 of 12 OVCA cell lines with the addition of FWGE. The reduction of IC_{50} was also found in the other 3 cell lines, although the increase in sensitivity did not reach statistical significance. Interestingly, cisplatin resistance, a surrogate marker of resistance to a broad range of cytotoxic chemotherapeutic agents, did not correlate with sensitivity to FWGE. This suggests that the biologic determinants of resistance to cytotoxic agents may differ from those that influence FWGE responsiveness.

We have also identified genes and molecular signaling pathways associated with FWGE OVCA activity. Expression of more than 2,000 genes (FDR < 0.2) and 27 pathways ($P < 0.05$) was associated with OVCA in vitro sensitivity to FWGE. These pathways included hedgehog signaling, activin A signaling regulation, and regulation of G_1/S transition. Interestingly, expression of 13 (48%) of 27 these pathways was also associated with in vitro sensitivity of a panel of 59 cancer cell lines subjected to treatment with a proposed active ingredient of FWGE, 2,6-dimethoxy-*p*-benzoquinone. These pathways included several cell cycle control pathways (including control of chromosome condensation in prometaphase, G_1/S transition, and never in mitosis-A-related kinase activity), control of apoptosis and cell survival, cytoskeleton remodeling, TGF-β signaling, and granzyme A and B signaling.

Our findings, related to FWGE activity in OVCA, are consistent with those of Mueller et al,[7] who demonstrated FWGE activity against a single OVCA cell line, A2780. Our data demonstrated that A2780 (and Chi) exhibits the highest levels of FWGE sensitivity. Fermented wheat germ extract has been shown to have potent in vitro cytotoxicity against leukemia, melanoma, and cancers of the breast, colon, testicle, head and neck, cervix, stomach, thyroid, and brain.[7,8,14,15]

We have identified an important role for cell cycle regulatory genes in the FWGE response, consistent with findings of other groups who have reported that FWGE attenuates the progression from G_2-M to G_0-G_1 phase of the cell cycle and reduces ribonucleotide reductase activity, a key enzyme in DNA synthesis.[9,16] Our molecular pathway findings are highly consistent with previous studies that have proposed mechanisms of FWGE action. Fermented wheat germ extract has previously been shown to induce apoptosis through poly(ADP-ribose) polymerase (PARP) cleavage in T-cell leukemia tumor cells, with subsequent prevention of DNA repair.[8] In our analysis, expression of PARP-1 and PARP-12 was associated with FWGE activity in OVCA cell lines (FDR < 0.15). Furthermore, PARP-1 is a known substrate for the proteolytic action of the granzyme suicidal proteases.[17] We identified both granzyme A and B signaling to be associated with cancer cell line sensitivity to both FWGE and 2,6-dimethoxy-*p*-benzoquinone. Importantly, granzyme A (also named cytolytic T-cell– and natural killer cell–specific trypsin-like serine protease) is known to induce caspase-independent apoptosis via a distinctive form of DNA damage: single-stranded DNA nicking.[18] Furthermore, the granzyme B/perforin pathway has been proposed as the predominant mechanism for immune-mediated apoptosis.[19] Fermented wheat germ extract has also been reported to act by enhancing the activity of the immune system by stimulating NK-cell activity (by reducing major histocompatibility complex I molecule expression), enhancing tumor necrosis factor secretion of the macrophages, and increasing intercellular adhesion molecule 1 molecule expression on the vascular endothelial cells; all of these lead to apoptosis of tumor cells.[5,20–22]

Our data demonstrate that FWGE increases OVCA cell response to cisplatin, which is consistent with previous work,

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which has demonstrated additive to synergistic drug interaction between FWGE and 5-fluorouracil, oxaliplatin, and irinotecan.[7] Our data shed provocative insights into associations between expression of PARP pathway genes and FWGE OVCA effect. In light of accumulating data demonstrating the utility of PARP inhibition in the clinical management of patients with particularly homologous recombination deficient OVCA, our findings underscore the potential utility of FWGE as an adjunct to current cytotoxic and biologically targeted therapeutic modalities.

The data we present enable us to conclude that FWGE has in vitro activity against OVCA cells, that it may increase the effect of cisplatin, and that its activity is influenced by the expression of distinct molecular signaling pathways. In light of these findings, it is possible to speculate that FWGE may have similar beneficial effects on OVCA cells in vivo, inducing apoptosis when used alone or increasing platinum-induced apoptosis, when used as a therapeutic agent for women affected by the disease. Clinical experience with FWGE suggests that it is well tolerated without significant adverse effects when taken by patients with melanoma and colon cancer.[10,11] Few data exist on the role of FWGE as a cancer-preventative agent, although the associations that we have observed between OVCA cell FWGE sensitivity and expression of key molecular signaling pathways, as well as its established tolerability, do make it a potentially attractive preventative agent. Although it is appealing to speculate that such FWGE-induced effects may influence OVCA risk and/or cancer clinical response rates to cytotoxic therapy, duration of response, or even overall survival, such possibilities are yet to be determined. However, they do underscore the potential need for clinical trials that would evaluate FWGE as both a preventative and therapeutic agent.

In conclusion, FWGE has significant antiproliferative effects on OVCA cell lines and may enhance the effect of cisplatin-induced cell death. Our genome-wide expression analysis supports the view that FWGE activity includes influences on cell cycle control, DNA repair, and immune function. Our findings demonstrate the value of FWGE as a natural product, with anticancer properties, which may also enhance the activity of existing therapeutic agents.

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