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Characterizing the efficacy of fermented wheat germ extract against ovarian cancer and defining the genomic basis of its activity

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Abstract

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

Methods—In this study, we investigated the activity of Avemar[®], a natural, non-toxic, 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 anti-proliferative effects against 12 human OVCA cell lines and potentiated cisplatin-induced apoptosis. Pearson's correlation of FWGE sensitivity and gene expression data identified 2,142 genes (FDR<0.2) representing 27 biological 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 chemo-sensitivity 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 anti-cancer 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.

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Keywords

Fermented wheat germ extract; Avemar; ovarian cancer; apoptosis; cisplatin

Introduction

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 intra-peritoneal 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 OVCA often demonstrate cross-resistance to the majority of 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 chemo-resistance is a critical determinant of survival for women with OVCA, and it is generally accepted that patients lose their battle with the disease when chemo-resistance 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, non-toxic 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 in Hungary in the early 1990s.⁵ It is produced by extraction of wheat germ from flour, fermentation of the extract, separation of the fermentation liquid, microencapsulation, drying, and granulation. 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 biological properties.⁶ FWGE 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 melanoma¹⁰ and colorectal cancer.¹¹

Despite reported data that FWGE induces apoptosis and has significant anti-tumor 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. 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.⁷

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, Virginia (SKOV3); the European Collection of Cell Cultures, Salisbury, England (A2780CP, A2780S); Kyoto University, Kyoto, Japan (CHI, CHICisR, M41, M41CSR, 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, Dept of OB/GYN, Division of GYN Oncology, Duke University, Durham, NC (OVCAR8, CAOV2, HAYA8). 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% nonessential amino acids (HyClone, Hudson, NH). Mycoplasma testing was performed every 6 months following manufacturer's protocol (Lonza, Rockland, ME).

Drugs and Reagents

FWGE was a gift from the manufacturer (American BioScience Inc, Blauvelt, NY). FWGE was stored as dried powder at 4°C. FWGE 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 FBS 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, $3\text{--}5 \times 10^4$ 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 $\mu\text{g/mL}$, 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 three 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 $(\text{control-treated}) / (\text{control-blank}) \times 100$. The concentration at which cell viability was decreased by 50% was used to define the IC_{50} . All experiments were performed three 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). Quality of the RNA was measured using an Agilent 2100 Bioanalyzer. 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 $\pm$ 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's correlation test was performed on individual gene expression and IC_{50} values using Significance Analysis of Microarray software. Probe sets with a FDR <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 chemo-sensitivity data for 2,6-dimethoxy-p-benzoquinone, a molecule postulated to contribute to FWGE's activity⁶ and 59 human cancer cell lines. In brief, chemo-sensitivity (GI_{50}) 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's 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 one 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 above. 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 above.

RESULTS

Effect of FWGE +/- Cisplatin on Ovarian Cancer 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 (Figure 1). The median IC_{50} was 244.7 $\mu\text{g/mL}$. The OVCA cell lines demonstrating extreme sensitivity to FWGE included SKOV3, which was most resistant (FWGE $IC_{50} = 561 \mu\text{g/mL}$) and A2780S, which was most sensitive (FWGE $IC_{50} = 105.7 \mu\text{g/mL}$).

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 of 12 (75%) 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 three 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; $p < 0.0001$). No antagonistic effects were observed (Figure 2).

Genes and Signaling Pathways Associated with FWGE Sensitivity

Pearson's correlation analysis of genome-wide expression data from the 12 OVCA cell lines and single-agent FWGE IC_{50} identified expression of 4,033 probe sets, representing 2,142 genes, to be associated with FWGE sensitivity (IC_{50}) ($FDR < 0.2$) (Table S1). 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 1), including cell cycle regulation of G1/S transition, apoptosis and survival/granzyme A signaling, and cytoskeleton remodeling.

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, publically available NCI-60 genomic and chemo-sensitivity data were analyzed for genes associated with 2,6-dimethoxy-p-benzoquinone sensitivity. This analysis identified 7,251 probe sets representing 3,839 genes (FDR < 0.2) (Table S2). GeneGo/MetaCore™ pathway analysis of genes/probe sets associated with 2,6-dimethoxy-p-benzoquinone sensitivity identified 267 pathways (FDR < 0.05) (Table S3).

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 G1/S transition, cell cycle/role of Nek in cell cycle regulation, cytoskeleton remodeling, cytoskeleton remodeling/reverse signaling by ephrin B, cytoskeleton remodeling/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 2). That is, 13/27 (48%) of 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, non-toxic fermented wheat germ extract, Avemar®, exhibits anti-proliferative and cytotoxic effects when applied on 12 different human OVCA cell lines. FWGE resulted in a significant reduction in OVCA survival after treating for 72 hours in all 12 cell lines. Although IC₅₀ 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 IC₅₀ for the least sensitive OVCA cell line was lower than the estimated peak plasma concentration after oral intake of standard dose of 9 g/day 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 ovarian cancer. The observed differences in FWGE IC₅₀ 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 mRNA expression levels and the molecular signaling pathways therein represented, or post translational events that influence apoptotic thresholds (e.g. phosphorylation of the BAD protein¹³). 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.

FWGE resulted in the potentiation of the cisplatin-induced apoptosis in almost all OVCA cell lines. The IC₅₀ of cisplatin was decreased significantly ($p < 0.05$) in 9 of 12 OVCA cell lines with the addition of FWGE. The reduction of IC₅₀ 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 G1/S transition. Interestingly, expression of 13/27 (48%) of 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, G1/S transition, and NIMA-related kinase activity), control of apoptosis and cell survival, cytoskeleton remodeling, TGF-beta 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 ovarian cancer cell line, A2780. Our data demonstrated that A2780 (and Chi) exhibits the highest levels of FWGE sensitivity. FWGE 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 G2-M to G0-G1 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. FWGE 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 FWGE has also been reported to act by enhancing the activity of the immune system by stimulating NK cell activity (by reducing MHC I molecule expression), enhancing TNF secretion of the macrophages, and

increasing ICAM-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, which has demonstrated additive to synergistic drug interaction between FWGE and 5-FU, 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 ovarian cancer 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 ovarian cancer 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 side effects when taken by patients with melanoma and colon cancer.^{10,11} Little data exist on the role of FWGE as a cancer preventative agent, although the associations that we have observed between ovarian cancer cell FWGE sensitivity and expression of key molecular signaling pathways, as well as its established tolerability, does make it a potentially attractive preventative agent. Although it is appealing to speculate that such FWGE-induced effects may influence ovarian cancer 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 anti-proliferative 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 anti-cancer properties, that may also enhance the activity of existing therapeutic agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DISCLOSURES

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RESEARCH HIGHLIGHTS

- FWGE has significant anti-proliferative 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 2,142 genes, representing 27 biological pathways.
- The known safety and tolerability of FWGE supports the clinical evaluation of this natural product in patients with ovarian cancer.

Figure 1. FWGE inhibits ovarian cancer cell line proliferation

The concentration of FWGE required to decrease OVCA cell viability by 50% (IC₅₀) was determined using the CellTiter96® MTS assay kit. Experiments were performed three times, or the minimum number of times to ensure reproducibility. Cell lines are ranked from most sensitive to most resistant.

Figure 2. FWGE 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 IC₃₀ for each cell line. All experiments were performed a minimum of three times.

Table 1Effect of FWGE on cisplatin IC₅₀ on 12 ovarian cancer cell lines

Ovarian Cell Line	IC ₅₀ FWGE (µg/mL)	IC ₅₀ Cisplatin (µM)	IC ₅₀ Cisplatin-FWGE (µM)	P value
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

Abbreviation: FWGE, Fermented wheat germ extract.

Table 2

FWGE Pathways

No.	Pathway Name	Pathway FDR	Pathway p Value	No. Obj. from Data	No. Obj. 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 G1/S transition (part 1)	<0.07	6.88E-06	12	38
4	Development_TGF-beta 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-beta-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 beta-catenin in the absence WNT signaling	<0.13	2.03E-04	7	19
10	Development_Beta-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 methabolism	<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

No.	Pathway Name	Pathway FDR	Pathway p Value	No. Obj. from Data	No. Obj. in Pathway
27	Cytoskeleton remodeling_ Cytoskeleton remodeling	<0.31	2.01E-03	16	102

Gene cutoff: FDR<0.2; pathway cutoff: FDR<0.05.

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 G1/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

Abbreviation: FWGE, Fermented wheat germ extract.