Systems Pharmacology Study of the Anticervical Cancer Mechanisms of FDY003

Ho-Sung Lee¹,², In-Hee Lee¹, Kyungrae Kang², Sang-In Park³, Tae-Wook Kwon², Seung-Joon Moon², Chol Hee Lee², and Dae-Yeon Lee¹,²

Abstract
Increasing data support that herbal medicines are beneficial in the treatment of cervical cancer; however, their mechanisms of action remain to be elucidated. In the current study, we used a systems pharmacology approach to explore the pharmacological mechanisms of FDY003, an anticancer herbal formula comprising Lonicera japonica Thunberg, Artemisia capillaris Thunberg, and Cordyceps militaris (Linn.) Link, in the treatment of cervical cancer. Through the pharmacokinetic assessment of absorption-distribution-metabolism-excretion characteristics, we found 18 active compounds that might interact with 106 cervical cancer-related targets responsible for the pharmacological effects. FDY003 targets were significantly associated with gene ontology terms related to the regulation of cellular behaviors, including cell proliferation, cell cycle processes, cell migration, cell apoptosis, cell death, and angiogenesis. The therapeutic targets of the herbal drug were further enriched in various oncogenic pathways that are implicated in the tumorigenesis and progression of cervical cancer, including the phosphatidylinositol 3-kinase, mitogen-activated protein kinase, focal adhesion, human papillomavirus infection, and tumor necrosis factor signaling pathways. Our study provides a systematic approach to explore the anticancer properties of herbal medicines against cervical cancer.

Keywords
herbal medicine, systems pharmacology, cervical cancer, anticancer agents, molecular mechanisms of pharmacological action

Received: July 31st, 2020; Accepted: November 7th, 2020.

Introduction
Cervical cancer is one of the most common malignancies and causes of cancer-related death in women worldwide.¹ Abnormalities in the regulation of key oncogenes and tumor-suppressor genes and their relevant oncogenic signaling pathways, which include the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)-Akt, focal adhesion, human papillomavirus (HPV) infection, tumor necrosis factor (TNF), and forkhead box O (FoxO) pathways, are implicated in the tumorigenesis and progression of cervical cancer.²,³ Chemotherapy and molecular targeted therapy are major pharmacological strategies for the treatment of this cancer.⁴ However, chemotherapeutic drugs can negatively impact the quality of life (QOL) of cancer patients due to undesirable side effects, such as digestive and gastrointestinal symptoms, fatigue, immunosuppression and myelosuppression, and cardiotoxicity.⁵ Moreover, most selective targeted agents have limited pharmacological activity in a variety of dysregulated cancer signaling pathways.⁶ These concerns highlight the requirement for safe and effective anticancer therapies that target diverse oncogenic signaling pathways simultaneously. Herbal medicines, which have a multi-compound, multitarget, and multipathway mode of action, have gained increasing attention because they exhibit potent anticancer effects by targeting multiple genes/proteins and pathways involved in tumorigenesis and cancer development while having fewer side effects than chemotherapeutic or targeted drugs.⁷,⁸ Clinical studies have reported the safety of herbal medicines and their efficacy in improving the survival rate as well as the QOL of cancer patients.⁹,¹⁰ FDY003 is a herbal formula comprising 3 herbal medicines (ie, Lonicera japonica Thunberg [LjT], Artemisia capillaris Thunberg [AcT], and Cordyceps militaris (Linn.) Link [Cm]) that possess anticancer properties.¹¹-²⁰ FDY003 inhibits cancer cell proliferation and survival and induces apoptosis in cancer cells in vitro and in vivo.²¹ FDY003 exerts therapeutic effects by modulating the activities of key regulators in the apoptotic signaling pathway, such as Bcl-2-associated X protein (Bax) and caspase-3, in colorectal cancer cells.²² However, the anticancer activities of FDY003 in cervical
cancer and the underlying system-level therapeutic mechanisms have not been fully understood.

Systems pharmacology is a research field that integrates various scientific areas such as medicine, pharmacology, and computational systems biology to elucidate the complex mechanisms of various diseases and develop effective therapeutic strategies for disease treatments at the systems level. This integrative approach has proven useful for investigating the pharmacological mechanism of action of herbal medicines. Systems pharmacology has been widely utilized to explore the therapeutic properties of herbal medicines by identifying the active compounds and their targets and to unravel system-level mechanisms coordinated by the complex interactions between them. This study aimed to explore the anticancer mechanisms of FDY003 based on a systems pharmacology approach.

Materials and Methods

Cell Culture

HeLa human cervical cancer cells were obtained from the Korean Cell Line Bank (Seoul, Korea). The cells were cultured in Dulbecco’s modified Eagle’s medium (WELGENE, Daegu, Korea) supplemented with 10% fetal bovine serum, and 100 U/mL penicillin, and 100 µg/mL of streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) in a humidified atmosphere containing 5% carbon dioxide (CO2) at 37 °C.

FDY003 Preparation

All raw herbal constituents of FDY003, approved by the Korea Ministry of Food and Drug Safety (Seoul, Korea), were obtained from Green Myeong-poem Pharm. (Namyangju, Korea). Dried plant materials of LjT (4.16 g), AcT (6.25 g), and Cm (6.25 g) were ground, mixed, and then reflux-extracted with 70% ethanol (500 mL) at 80 °C for 3 hours. After filtration through a 1-μm pore filter (Hyundai Micro, Seoul, Korea), the herbal extract was purified using 80% and 90% ethanol and freeze-dried at −80 °C. The freeze-dried samples were stored at −20 °C and dissolved in distilled water before use.

Cell Viability Assay

Cell viability was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously. In brief, HeLa cells were seeded into 48-well plates (5.0 × 10⁴ cells/well) and treated with varying doses of FDY003 at 37 °C in the presence of 5% CO₂ for 48 hours. Then, 200 µL of MTT (Sigma-Aldrich, St. Louis, MO, USA) was added to each well, and the cells were incubated for 2 hours. The purple, soluble formazan crystals were dissolved using dimethyl sulfoxide. The absorbance at 550 nm was measured using an Epoch 2 microplate reader (BioTek, Winooski, VT, USA).

Investigation of the Active Compounds in FDY003

We surveyed the phytochemical compounds in the herbal constituents of FDY003 (ie, LjT, AcT, and Cm) using information extracted from databases containing comprehensive pharmacological information relevant to herbal medicines such as the Traditional Chinese Medicine Systems Pharmacology (TCMSP) and CancerHSP databases. To identify the active compounds of FDY003, we evaluated absorption, distribution, metabolism, and excretion (ADME) properties, including oral bioavailability (OB), Caco-2 permeability, and drug-likeness (DL). For individual phytochemical constituents in the 3 herbal medicines in FDY003, OB is the fraction of an administered dose of a substance that reaches the systemic circulation and becomes available at the site of therapeutic action. Caco-2 permeability is the rate of flux of a compound across polarized monolayers of Caco-2 human colon epithelial cancer cells and is widely used to assess and predict the intestinal drug absorption capacity and extent of drug molecules. A compound is considered permeable and to be absorbed in the intestinal epithelium if its Caco-2 permeability is equal to or greater than −0.4. DL is a parameter considered for drug design and development to evaluate the drug potential of prospective chemical compounds based on their pharmacokinetic and pharmaceutical features. Compounds with OB ≥30%, Caco-2 permeability ≥-0.4, and DL ≥0.18 were regarded as active compounds, as described previously.

Exploration of the Targets of Active Compounds in FDY003

Target genes/proteins of the active compounds in FDY003 were explored from Search Tool for Interactions of Chemicals (STITCH) 5, SwissTargetPrediction, PharmMapper, and Similarity Ensemble Approach (SEA). In silico models such as the weighted ensemble similarity (WES) algorithm and systematic drug targeting tool (SysDt) were also applied for the target investigation, as described previously. Biological information for the targets was verified and standardized using Uniprot. Cervical cancer-related human genes/proteins were retrieved from various relevant databases, including DisGeNET, DrugBank, GeneCards, Human Genome Epidemiology Navigator, Online Mendelian Inheritance in Man, Pharmacogenomics Knowledge for Personalized Medicine, Therapeutic Target Database, and the Comparative Toxicogenomics Database using the search term “cervical cancer”, while setting the species to Homo sapiens.

Network Construction

Herb-compound (H-C) and compound-target (C-T) networks were generated by connecting the herbal medicines and their active compounds, and the active compounds and their potential targets, respectively. A target-pathway (T-P) network was constructed by connecting the targets and the signaling pathways they are involved in. A protein-protein interaction (PPI) network was built using the highest-confidence human protein
interaction pairs (confidence score ≥0.9) among the targets obtained from the STRING database (version 11.0). Cytoscape software was used for network visualization. In the networks, nodes refer to the herbal medicines, bioactive compounds, targets, or pathways, and edges (or links) indicate their interactions. The degree of a node is defined as the number of its edges in a network.

Survival Analysis

The Kaplan-Meier Plotter was used to analyze the correlation between the expression levels of key targets of FDY003 and the survival rates of cervical cancer patients.

Contribution Index Analysis

To investigate the contribution of individual active phytochemicals to the anticancer properties of FDY003, a contribution index (CI) was obtained on the basis of the network-based efficacy, using the following equations, as described previously:

\[
NE(j) = \sum_{i=1}^{n} d_i
\]

\[
CI(j) = \frac{\sum_{i=1}^{m} c_i \times NE(i)}{\sum_{i=1}^{n} c_i \times NE(i)} \times 100\%
\]

where \(d_i\) is the degree of protein \(i\) targeted by compound \(j\); \(n\) is the number of targets of compound \(j\); \(m\) is the number of compounds, and \(c_i\) is the number of previous studies regarding both cervical cancer and compound \(i\). The term “cervical cancer” and the common names of individual compounds were used as search terms to survey the literature related to cervical cancer and the active compounds. The papers in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) that contained the search terms in the title or abstract were counted. If the sum of CIs of the top \(N\) chemical components was larger than 85%, those \(N\) components were determined as the main contributors for the pharmacological activity of FDY003, as described previously.

Functional Enrichment Analysis

Functional enrichment analysis of the FDY003 targets was performed using the g:Profiler and Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Molecular Docking Analysis

To verify the binding between the key targets and bioactive compounds of FDY003, we performed molecular docking analysis. For this purpose, the molecular structures of chemical compounds were acquired from the PubChem database, and the protein structures of the targets were obtained from the RCSB Protein Data Bank database. Then, docking scores for the binding interactions between the compounds and targets were calculated using Autodock Vina. In general, a docking score of less than –5.0 indicates that a compound may have good binding activity with a certain target, and a lower score suggests a stronger and more stable binding interaction.

Results

We conducted a systems pharmacology-based exploration of the therapeutic mechanism of FDY003 (Figure 1). First, we extensively surveyed multiple TCM-associated databases for information on the chemical compounds of the 3 herbal medicines constituting FDY003 (Figure 1). Next, we evaluated the ADME parameters of the phytochemicals of FDY003 to identify potentially active compounds that may play major roles in the anticancer effects of this medicine (Figure 1). Then, target genes/proteins of the active compounds were explored through in silico analysis of compound-protein interactions (Figure 1). Finally, the comprehensive information on FDY003 was merged into H-C, C-T, and T-P networks, and the pharmacological mechanisms of the herbal formula were explored at the systems level (Figure 1).

Anticancer Effects of FDY003 on Cervical Cancer Cells

To assess the pharmacological effects of FDY003 on cervical cancer, HeLa human cervical cancer cells were treated with FDY003 for 48 hours, after which their viability was evaluated. FDY003 treatment significantly decreased HeLa cell viability (Supplemental Figure S1), suggesting that the herbal formula exhibits anticancer activity against cervical cancer cells.

Chemical Compounds in FDY003

The chemical compounds of the 3 herbal medicines (i.e., LtT, AcT, and Cm) constituting FDY003 were retrieved from TCM-associated databases such as TCMSP and CancerHSP. In total, 324 FDY003 compounds were obtained (237, 56, and 40 compounds for LtT, AcT, and Cm, respectively) after duplicate removal (Supplemental Table S1).

Active Compounds in FDY003

In silico modeling of ADME properties has proven useful in the exploration of active compounds primarily responsible for the therapeutic effects of a given drug. To screen for potentially bioactive compounds in FDY003, ADME parameters of the chemical compounds in FDY003 were evaluated. Potential active compounds were identified based on the following criteria: OB ≥30%, Caco-2 permeability ≥–0.4, and DL ≥0.18, as described previously. Some compounds that did not satisfy the criteria but were abundantly present in the 3 herbal constituents of FDY003 and reportedly have potent pharmacological properties were also considered as bioactive compounds. In total, 20 potentially active compounds were identified (Supplemental Table S2).
Targets of the Active Compounds in FDY003

To investigate the pharmacological targets of FDY003, we used various in silico tools to predict chemical-protein interactions, including STITCH 5,37, SEA,41 SwissTargetPrediction,38,39 and PharmMapper.40 In addition, we employed the SysDt43 and WES algorithms42 for target investigation, as previously described.44-50 In total, 196 targets were identified for 18 active compounds in FDY003 (Supplemental Table S3). For 2 compounds, loniceracetalides B_qt and demethoxycapillarisin, no potential targets were obtained.

Systems-Level Pharmacological Mechanisms of FDY003

To analyze the systems-level mechanisms of FDY003 based on a systems pharmacology approach, an herb-compound-target (H-C-T) network was built by connecting the herbal medicines with their active compounds and these in turn with their targets (Figure 2). The H-C-T network for FDY003 consisted of 217 nodes (3 herbal medicines, 18 bioactive compounds, and 196 targets) and 353 edges (Figure 2). Then, for a network-level exploration of the pharmacological characteristics of FDY003, a C-T network (124 nodes and 195 links) was built by connecting the active compounds and their cervical cancer-associated targets (Figure 3, Supplemental Table S3). The active compounds quercetin (degree = 117), luteolin (degree = 48), cordycepin (degree = 39), kaempferol (degree = 37), eriodictiol (flavanone) (degree = 23), β-sitosterol (degree = 19), and isorhamnetin (degree = 18) had the largest numbers of cervical cancer-related targets (Figure 3), which suggests that they may be the major active compounds responsible for the therapeutic actions of FDY003. Furthermore, 36 cervical cancer-associated genes/proteins were targeted by 2 or more active compounds (Figure 3), supporting the multicompound, multitarget polypharmacological features of FDY003.

To explore the biological characteristics of the targets at the network level, we generated a PPI network (92 nodes and 226 edges) composed of the paired interactions between the cervical cancer-associated targets of FDY003 (Figure 4). Then, we investigated the hub nodes, high-degree nodes that are shown to play key roles in diverse cellular functions,71,72 in the PPI network. In this study, a node was defined as a hub if its degree was equal to or higher than twice the average degree of all nodes in a network.73,74 Among the cervical cancer-associated FDY003 targets, TP53 (degree = 29), SRC (degree = 21), AKT1 (degree = 18), VEGFA (degree = 18), JUN (degree = 15), EGFR (degree = 14), MAPK8 (degree = 12), ESR1 (degree = 12), and AKR1C3 (degree = 11) were hubs, suggesting that they may be important targets involved in the pharmacological effects of FDY003 on cervical cancer cells (Figure 4). Functional loss of the tumor suppressor p53 (encoded by

Figure 1. A schematic illustration for the workflow of the systems pharmacology-based exploration of the therapeutic mechanisms of FDY003 in the treatment of cervical cancer.
TP53, a crucial regulator of cell proliferation, apoptosis, metabolism, and cell cycling, may facilitate the tumorigenesis and progression of cervical cancer, and its restoration or (re-)activation has been suggested as a promising oncological therapy.75-77 Src (encoded by SRC) is a nonreceptor tyrosine kinase that is dysregulated in many types of cancer, including

Figure 2. The herb-compound-target network of FDY003. Green and red nodes refer to the 3 herbal constituents of FDY003 and their 18 active compounds, respectively. Ovals refer to the 196 targets of the active compounds; those closely related to the tumorigenesis and progression of cervical cancer are colored in blue.

Figure 3. The compound-target network of FDY003. Red and blue nodes represent the 18 active compounds in FDY003 and their 106 cervical cancer-associated targets, respectively.
cervical cancer, and it plays an important role in cancer progression by promoting proliferation, migration, and invasion of cervical cancer cells. AKT (encoded by AKT1), epidermal growth factor receptor (EGFR; encoded by EGFR), and c-Jun (encoded by JUN) exhibit diverse tumorigenic activities, and their targeting may enhance therapeutic sensitivity to chemotherapy and radiotherapy in cervical cancer cells. Vascular endothelial growth factor A (VEGF-A; encoded by VEGFA) functions as a key modulator of cervical cancer cell growth, angiogenesis, and metastatic behavior. Activation of estrogen receptor α (encoded by ESR1) is required for the onset and malignant progression of cervical cancer. c-Jun N-terminal kinase 1 (JNK1; encoded by MAPK8) is an important mediator of apoptosis of cervical cancer cells in response to anticancer drug treatment. Aldo-keto reductase family 1 member C3 (AKR1C3; encoded by AKR1C3) is involved in the regulation of migratory and invasive capabilities of cervical cancer cells and is associated with higher recurrence rates and poorer survival outcomes in cervical cancer patients.

Among the hub targets of FDY003, survival analysis further showed that high expression of TP53, ESR1, and AKR1C3 and low expression of AKT1, VEGFA, JUN, and EGFR were associated with a higher survival rate of cervical cancer patients, suggesting that these compounds might be the main contributors to the therapeutic activities of FDY003 in cervical cancer treatment.

Collectively, the results suggested that FDY003 has a complex network-level action mechanism.

Functional Enrichment Analysis of the FDY003 Network

To understand the functional properties of the cervical cancer-associated targets of FDY003, they were subjected to gene ontology (GO) enrichment analysis. The targets are significantly involved in the regulation of cellular behaviors, including cell proliferation, the cell cycle process, cell migration, cell apoptosis, cell death, and angiogenesis (Supplemental Figure S3), which provides insights into the anticancer mechanisms of FDY003.

The dysregulation of various oncogenic pathways is involved in the tumorigenesis and progression of diverse cancer types. To explore the signaling mechanisms of FDY003, KEGG pathway enrichment analysis was conducted for the cervical cancer-related targets of the herbal formula (Figure 6, Supplemental Figures S3 and S4). The targets were found to be involved in various pathways associated with the pathogenesis of cervical cancer, including “pathways in cancer,” “PI3K-Akt signaling pathway,” “MAPK signaling pathway,” “focal adhesion,” “human papillomavirus infection,” “TNF signaling pathway,” “steroid hormone biosynthesis,” “viral carcinogenesis,” “apoptosis,” “cellular senescence,” and others.

Figure 4. The protein-protein interaction network for cervical cancer-related targets of FDY003. Nodes indicate the cervical cancer-related targets of the active compounds in FDY003.
“estrogen signaling pathway,” “FoxO signaling pathway,” “PD-L1 expression and PD-1 checkpoint pathway in cancer,” “HIF-1 signaling pathway,” “Wnt signaling pathway,” “cell cycle,” “ErbB signaling pathway,” “p53 signaling pathway,” “VEGF signaling pathway,” and “prolactin signaling pathway.” Previous studies have shown the central roles of these pathways in the tumorigenesis and progression of cervical cancer. Dysfunction of the PI3K-Akt, MAPK, focal adhesion, erythroblastic leukemia viral oncogene homolog (ErbB), and hypoxia-inducible factor 1 (HIF-1) pathways has been strongly implicated in tumor initiation and development.

---

**Figure 5.** Survival analysis of the cervical cancer-associated hub targets of FDY003. Kaplan-Meier curves for overall survival of cervical cancer patients according to the expression of indicated targets.

**Figure 6.** The herb-compound-target-pathway network of FDY003. Green and red nodes indicate the 3 herbal medicines comprising FDY003 and their 18 active compounds, respectively. Blue and orange nodes indicate the cervical cancer-associated targets of the active compounds and the signaling pathways enriched with the corresponding targets, respectively.
in cervical cancer. Dysregulated cellular processes, including senescence, apoptosis, and cell cycle regulation, are key pathological mechanisms in cervical cancer. High endogenous levels of estradiol, an important estrogen steroid hormone, are associated with an elevated risk of cervical cancer, and activation of its downstream estrogen signaling pathway contributes to the malignant development of cervical cancer. The upregulation of programmed death-ligand 1 (PD-L1) expression is negatively correlated with survival outcomes in cervical cancer patients, and anti-PD-L1 therapies have been suggested as potentially effective therapeutic strategies for cervical cancer treatment. The prolactin pathway has an antiproliferative role, and its overactivity is closely associated with enhanced cancer cell survival in cervical cancer. The p53 and FoxO pathways are implicating in the modulation of cell proliferation, migration, and invasion capacities induced in cervical cancer cells by anticancer agents. The TNF signaling pathway acts as a major regulator of inflammation and is associated with HPV-related cervical cancer progression. The Wnt signaling pathway is crucially involved in the regulation of cell differentiation, proliferation, and stem cell-like properties in cervical cancer cells. Aberrant regulation of the VEGF signaling pathway contributes to cervical cancer progression by promoting angiogenesis and tumor metastasis.

Pathway mapping analysis of the FDY003 targets suggested that FDY003 may exert its pharmacological activities by synergistically acting on multiple genes/proteins involved in various signaling pathways associated with the tumorigenesis and progression of cervical cancer (Supplemental Figure S5), which supports the multicomponent, multitarget, multipathway pharmacological properties of the herbal formula.

Functional association analysis of the cervical cancer-related FDY003 targets was further performed using GeneMANIA. The analysis revealed that 32.7% and 37.5% of the targets may be co-expressed and engaged in physical interactions, respectively (Supplemental Figure S6), implying that they may have similar cellular functions and characteristics.

Taken together, these results suggested that FDY003 may exhibit pharmacological activities by regulating multiple cervical cancer-related pathways and relevant biological processes.

**Molecular Docking Analysis**

To validate the binding activity of key compounds of FDY003 to the therapeutic targets, we conducted molecular docking analysis for the bioactive compounds of the herbal drug and their hub targets (see Materials and Methods section). We found that 96.9% (157 out of 162) of interaction pairs between the compounds and the targets had docking scores of less than −5.0, suggesting their potential pharmacological binding abilities (Supplemental Figures S7 and S8).

**Discussion**

Cervical cancer remains among the most widely prevalent malignancies in women globally. Herbal medicines have gained increased interest for cancer treatment owing to their potent anticancer properties together with relatively low toxicity and few side effects. By employing a systems pharmacology approach, we investigated the therapeutic mechanisms underlying the pharmacologic effects of FDY003 in cervical cancer treatment. The following are our major findings: (1) 18 potential bioactive compounds in FDY003 may exert their pharmacological action by targeting 106 cervical cancer-related targets; (2) FDY003 targets were significantly associated with GO terms related to the regulation of cellular behaviors, including cell proliferation, cell cycle progression, cell migration, cell apoptosis, cell death, and angiogenesis; and (3) the therapeutic targets of the herbal medicine are involved in diverse oncogenic signaling cascades, including the PI3K-Akt, MAPK, focal adhesion, HPV infection, and TNF signaling pathways, which are involved in tumorigenesis and progression in cervical cancer.

The herbal and chemical components of FDY003 have been previously shown to possess antitumor properties. Lignans, AcT, Cm, genkwanin, and carpillarisin have shown antiproliferative and apoptotic effects in cervical cancer cells. Kaempferol, luteolin, and β-sitosterol can induce apoptosis while inhibiting proliferation and invasiveness of cervical cancer cells by targeting various oncogenic kinases and signaling pathways. Quercetin exerts anticancer effects in cervical cancer cells by regulating their dysregulated behaviors in proliferation, cell cycle progression, survival, apoptosis, migration, and invasion. Isorhamnetin suppresses the proliferation of cervical cancer cells by inducing cell cycle arrest in the G2/M phase. Cordycepin stimulates apoptotic cell death and interferes with the cell cycle through the generation of reactive oxygen species and the repression of cell cycle regulators in cervical cancer cells, and sensitizes cervical cancer cells to radiotherapy.

Collectively, these previous findings may provide a basis for the anticancer effects and the underlying pharmacological mechanisms of the herbal and chemical constituents of FDY003. The pharmacological activities of FDY003 in cancer treatment have been investigated previously. In human colorectal cancer cells in vitro, FDY003 exerted pharmacological effects by modulating the activities of important apoptosis regulators, including Bax, caspase-3, p21, and p53, thereby suppressing cell proliferation, while inducing apoptosis. The therapeutic effects of the herbal formula in vivo were confirmed in a xenograft model: mice engrafted with human colorectal cancer cells showed significant tumor regression upon FDY003 treatment. It is noteworthy that unlike irinotecan, a chemotherapeutic anticancer drug widely used in the clinic, FDY003 did not cause body weight loss in experimental animals during the treatment period, suggesting that the herbal medicine is tolerable. Further experimental investigation of the action mechanisms of FDY003 would advance the design of safe and effective herbal medicine-based anticancer therapeutic strategies.
In summary, we investigated the pharmacological mechanism of FDY003 at the systems level. Based on a systems pharmacology analysis, we identified 18 potentially active compounds in FDY003 that may interact with 106 cervical cancer-related targets. Functional enrichment analysis showed that the FDY003 targets were implicated with GO terms related to the regulation of cellular behaviors, including cell proliferation, cell cycle progression, cell migration, cell apoptosis, cell death, and angiogenesis and that they are involved in diverse pathways related to cervical cancer tumorigenesis and progression, including PI3K-Akt, MAPK, focal adhesion, HPV infection, and TNF signaling. Taken together, the results provide novel insights into the systems-level pharmacological mechanisms of FDY003 for cervical cancer treatment.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID ID

Dae-Yeon Lee https://orcid.org/0000-0002-3198-9881

Supplemental Material

Supplemental material for this article is available online.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69(1):7-34. doi:10.3322/caac.21551
2. Manzo-Merino J, Contreras-Paredes A, Vázquez-Ulloa E, Rocha-Zavaleta I, Fuentes-Gonzalez AM, Lizano M. The role of signaling pathways in cervical cancer and molecular therapeutic targets. Arch Med Res. 2014;45(7):525-539. doi:10.1016/j.arcmed.2014.10.008
3. Zhang L, Wu J, Ling MT, Zhao L, Zhao KN. The role of the PI3K/Akt/mTOR signalling pathway in human cancers induced by human papillomaviruses. Mol Cancer. 2015;14(1):1-13. doi:10.1186/s12943-015-0361-x
4. Kumar I, Harish P, Malik PS, Khurana S. Chemotherapy and targeted therapy in the management of cervical cancer. Curr Probl Cancer. 2018;42(2):120-128. doi:10.1016/j.ccurprobcancer.2018.01.016
5. Oun R, Moussa YE, Wheate NJ. The side effects of platinum-based chemotherapy drugs: a review for chemists. Dalton Trans. 2018;47(19):6645-6653. doi:10.1039/C8DT00838H
6. Zimmermann GR, Lehár J, Keith CT. Multi-target therapeutics: when the whole is greater than the sum of the parts. Drug Discov Today. 2007;12(1-2):34-42. doi:10.1016/j.drudis.2006.11.008
7. Ohnishi S, Takeda H. Herbal medicines for the treatment of cancer chemotherapy-induced side effects. Front Pharmacol. 2015;6(Suppl. 5):1-5. doi:10.3389/fphar.2015.00014
8. Poornima P, Kumar JD, Zhao Q, Blunder M, Efferth T. Network pharmacology of cancer: from understanding of complex interactomes to the design of multi-target specific therapeutics from nature. Pharmacol Res. 2016;111:290-302. doi:10.1016/j.phrs.2016.06.018
9. Yin SY, Wei WC, Jian FY, Yang NS. Therapeutic applications of herbal medicines for cancer patients. Evid Based Complement Alternat Med. 2013;2013:1-15. doi:10.1155/2013/302426
10. Zhu L, Li L, Li Y, Wang J, Wang Q. Chinese herbal medicine as an adjunctive therapy for breast cancer: a systematic review and meta-analysis. Evid Based Complement Alternat Med. 2016;2016:1-17. doi:10.1155/2016/9469276
11. Feng G, Wang X, You C, et al. Antiproliferative potential of Artemisia capitulla polysaccharide against human nasopharyngeal carcinoma cells. Carbohydr Polym. 2013;92(2):1040-1045. doi:10.1016/j.carbpol.2012.10.024
12. Rao YK, Fang SH, Wu W-S, Tseng Y-M. Constituents isolated from Cordyceps militaris suppress enhanced inflammatory mediator's production and human cancer cell proliferation. J Ethnopharmacol. 2010;131(2):363-367. doi:10.1016/j.jep.2010.07.020
13. Zhou Q, Zhang Z, Song L, et al. Cordyceps militaris fraction inhibits the invasion and metastasis of lung cancer cells through the protein kinase B/glycogen synthase kinase 3β/β-catenin signaling pathway. Oncol Lett. 2018;16(6):6930-6939. doi:10.3892/ol.2018.9518
14. Jang E, Kim SY, Lee NR, et al. Evaluation of antitumor activity of Artemisia capitulla extract against hepatocellular carcinoma through the inhibition of IL-6/STAT3 signaling axis. Oncol Rep. 2017;37(1):526-532. doi:10.3892/ol.2016.5283
15. Kim J, Jung KH, Yon HH, et al. Artemisia capitulla leaves inhibit cell proliferation and induce apoptosis in hepatocellular carcinoma. BMC Complement Altern Med. 2018;18(1):1-10. doi:10.1186/s12906-018-2217-6
16. Park KI, Park H, Nagappan A, et al. Polyphenolic compounds from Korean Lonicera japonica Thunb. induces apoptosis via AKT and caspase cascade activation in A549 cells. Oncol Lett. 2017;13(4):2521-2530. doi:10.3892/ol.2017.5771
17. Park HS, Park KI, Lee DH, et al. Polyphenolic extract isolated from Korean Lonicera japonica Thunb. induce G2/M cell cycle arrest and apoptosis in HepG2 cells: involvements of PI3K/Akt and MAPKs. Food Chem Toxicol. 2012;50(7):2407-2416. doi:10.1016/j.fct.2012.04.034
18. Yoo HS, Shin JW, Cho JH, et al. Effects of Cordyceps militaris extract on angiogenesis and tumor growth. Acta Pharmacol Sin. 2004;25(5):657-665.
19. Jin CY, Kim GY, Choi YH. Induction of apoptosis by aqueous extract of Cordyceps militaris through activation of caspases and inactivation of Akt in human breast cancer MDA-MB-231 Cells. J Microbiol Biotechnol. 2017;27(2):184-194.
20. Chen C, Wang ML, Jin C, et al. Cordyceps militaris polysaccharide triggers apoptosis and G(0)/G(1) cell arrest in cancer cells. J Asia Pac Entomol. 2015;18(3):433-438. doi:10.1016/j.aspen.2015.04.015
21. Lee D-Y, Lee I-H. FDY003 inhibits colon cancer in a COLO205 xenograft mouse model by decreasing oxidative stress. *Pharmacog Mag*. 2019;15(65):675-681. doi:10.4103/pm.pm_650_18

22. Hao DC, Xiao PG. Network pharmacology: a Rosetta stone for traditional Chinese medicine. *Drug Dev Res*. 2014;75(5):299-312. doi:10.1002/ddr.21214

23. Lee WY, Lee CY, Kim YS, Kim CE. The methodological trends of traditional herbal medicine employing network pharmacology. *Biomolecules*. 2019;9(8):362-15. doi:10.3390/biom9080362

24. Lee HS, Lee IH, Park SI, Lee DY. FDY003 inhibits colon cancer in a COLO205 xenograft mouse model by decreasing oxidative stress. *Front Pharmacol*. 2013;4:11481. doi:10.1038/srep11481

25. Wang CK, Craik DJ. Cyclic peptide oral bioavailability: lessons from the past. *Biopolymers*. 2016;106(6):901-909. doi:10.1002/bip.22878

26. Kono Y, Iwasaki A, Matsuoka K, Fujita T. Effect of mechanical agitation on cationic liposome transport across an unstirred water layer in Caco-2 cells. *BioPharm Bull*. 2016;39(8):1293-1299. doi:10.1248/bpb.16-00050

27. Tao W, Li B, Gao S, et al. CancerHSP: anticancer herbs database of systems pharmacology. *Sci Rep*. 2015;5(1):11481. doi:10.1038/srep11481

28. Yue SJ, Xin LT, Fan YC, et al. Herb pair Danggui-Honghua: mechanisms underlying blood stasis syndrome by system pharmacology approach. *Sci Rep*. 2017;7:40318. doi:10.1038/srep40318

29. Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. String 5: augmenting protein-chemical interaction networks with tissue and affinity data. *Nucleic Acids Res*. 2016;44(D1):D380-D384. doi:10.1093/nar/gkv1277

30. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res*. 2019;47(W1):W357-W364. doi:10.1093/nar/gkz382

31. Wang X, Shen Y, Wang S, et al. PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. *Nucleic Acids Res*. 2017;45(W1):W356-W360. doi:10.1093/nar/gkz374

32. Yang Y, Huang C, Su X, et al. Deciphering the multicomponent mechanism of anti- non-small cell lung cancer for Hedyotis diffusa.
Szklarczyk D, Gable AL, Lyon D, et al. String v11: protein association networks with increased coverage, datasets. *Nucleic Acids Res.* 2017;45(D1):D833-D839. doi:10.1093/nar/gkw943

Piñero J, Bravo A, Queralt-Rosina N, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res.* 2017;45(D1):D833-D839. doi:10.1093/nar/gkw943

Wishart DS, Feunang YD, Guo AC, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res.* 2018;46(D1):D1074-D1082. doi:10.1093/nar/gkx1037

Safran M, Dalah I, Alexander J, et al. GeneCards version 3: the human gene integrator. *Database.* 2010;2010:bao020. doi:10.1093/database/bao020

Yu W, Gwinn M, Clyne M, Yesupriya A, Khoury MJ. A navigator for human genome epidemiology. *Nat Genet.* 2004;5(2):101-113.

Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A. OMIM.org: online Mendelian inheritance in man (OMIM®), an online catalog of human genes and genetic disorders. *Nucleic Acids Res.* 2015;43(Database issue):D789-D798. doi:10.1093/nar/gku1205

Whirl-Carrillo M, McDonagh EM, Hebert JM, et al. Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther.* 2012;92(4):414-417. doi:10.1038/clpt.2012.96

Zhu F, Han B, Kumar P, et al. Update of TTD: therapeutic target database. *Nucleic Acids Res.* 2010;38(Database issue):D787-D791. doi:10.1093/nar/gkz1039

Davis AP, Grondin CJ, Johnson RJ, et al. The comparative toxicogenomics database: update 2019. *Nucleic Acids Res.* 2019;47(D1):D948-D954. doi:10.1093/nar/gky868

Zhuang Z, Wen J, Zhang L, et al. Can network pharmacology identify the anti-virus and anti-inflammatory activities of Shuanghuanglian oral liquid used in Chinese medicine for respiratory tract infection? *Eur J Integr Med.* 2020;37:101139. doi:10.1016/j.eujim.2020.101139

Zhang M, Yuan Y, Zhou W, et al. Network pharmacology analysis of Chaihu Lizhong Tang treating non-alcoholic fatty liver disease. *Comput Biol Chem.* 2020;86:107248. doi:10.1016/j.compbiolchem.2020.107248

Cho DY, Kim YA, Przytycka TM. Chapter 5: network biology approach to complex diseases. *PLoS Comput Biol.* 2012;8(12):e1002820. doi:10.1371/journal.pcbi.1002820

Jeong H, Mason SP, Barabási AL, Oltvai ZN. Lethality and centrality in protein networks. *Nature.* 2001;411(6833):41-42. doi:10.1038/35075138

Zhong J, Liu Z, Zhou X, Xu J. Synergic anti-pruritus mechanisms of action for the Radix sophorae Flavescentis and Fructus Caryophylli herbal pair. *Molecules.* 2017;22(9):1-13. doi:10.3390/molecules22091465

Zhu J, Yi X, Zhang Y, Pan Z, Zhong L, Huang P. Systems pharmacology-based approach to comparatively study the independent and synergistic mechanisms of DanHong injection and Naoxintong capsule in ischemic stroke treatment. *Evid Based Complement Alternat Med.* 2019;2019:1-17. doi:10.1155/2019/1056708

Węsierska-Gądek J. Targeting p53 as a promising therapeutic option for cancer by re-activating the wt or mutant p53’s tumor suppression. *Future Med Chem.* 2018;10(7):755-777. doi:10.4155/fmc-2017-0175

Hietanen S, Lain S, Krausz E, Blattner C, Lane DP. Activation of p53 in cervical carcinoma cells by small molecules. *Proc Natl Acad Sci U S A.* 2000;97(15):8501-8506. doi:10.1073/pnas.97.15.8501

Sun W, Zhao X, Lu Z, Guo Q. P53 introduction enhances chemotherapy-induced apoptosis in HeLa cells. *Transl Cancer Res.* 2018;7(4):1103-1111. doi:10.21037/tcr.2018.08.25

Al Moustafa AE, Yaseen A, Alachkar A, As hh khar A. Src inhibitors are promising therapy molecules for human cervical carcinomas. *Med Hypotheses.* 2011;77(5):812-814. doi:10.1016/j.mehy.2011.07.043
79. Sima N, Cheng X, Ye F, Ma D, Xie X, Lu W. The overexpression of scaffolding protein NEDD9 promotes migration and invasion in cervical cancer via tyrosine phosphorylated FAK and Src. *PLoS One*. 2013;8(9):e74594. doi:10.1371/journal.pone.0074594

80. Hou T, Xiao J, Zhang H, Gu H, Feng Y, Li J. Phosphorylated c-Src is a novel predictor for recurrence in cervical squamous cell cancer patients. *Int J Clin Exp Pathol*. 2013;6(6):1121-1127.

81. Yoshida T, Nishimura M, Mineda A, Kawakita T, Abe A, Irahara M. Growth inhibitory effect of the Src inhibitor dasatinib in combination with anticancer agents on uterine cervical adenocarcinoma cells. *Exp Ther Med*. 2017;14(5):2493-2499. doi: 10.3892/etm.2017.5061

82. Yasmeen A, Alachkar A, Gembacorti-Passerini C, Al Moustafa A-E. Locking Src/Abi tyrosine kinase activities regulate cell differentiation and invasion of human cervical cancer cells expressing E6/E7 oncoproteins of high-risk HPV. *J Oncol*. 2010;2010(530130):1-10. doi:10.1155/2010/530130

83. Liu C, Ding L, Bai L, et al. Folate receptor alpha is associated with cervical carcinogenesis and regulates cervical cancer cells growth by activating ERK1/2/c-Fos/c-Jun. *Biochem Biophys Res Commun*. 2017;491(4):1083-1091. doi:10.1016/j.bbrc.2017.08.015

84. Lu Z, Chen H, Zheng XM, Chen ML. Experimental study on the apoptosis of cervical cancer Hela cells induced by juglone through c-Jun N-terminal kinase/c-Jun pathway. *Asian Pac J Trop Med*. 2017;10(6):572-575. doi:10.1016/j.aptm.2017.06.005

85. Xiong H, Nie X, Zou Y, et al. Twist1 enhances hypoxia induced radioresistance in cervical cancer cells by promoting nuclear EGFR localization. *J Cancer*. 2017;8(3):345-353. doi:10.7150/jca.16607

86. Liu J, Sun Y, Zhang H, et al. Theanine from tea and its semi-synthetic derivative TBrC suppress human cervical cancer growth and migration by inhibiting EGFR/Met-Akt/NF-kB signaling. *Eur J Pharmacol*. 2016;791:297-307. doi:10.1016/j.ejphar.2016.09.007

87. Guo L, Wu H, Zhu J, et al. Genetic variations in the PI3K/Akt pathway predict platinum-based neoadjuvant chemotherapeutic sensitivity in squamous cervical cancer. *Life Sci*. 2015;143:217-224. doi:10.1016/j.lfs.2015.11.011

88. He C, Mao D, Hua G, et al. The Hippo/YAP pathway interacts with EGFR signaling and HPV oncoproteins to regulate cervical cancer progression. *EMBO Mol Med*. 2015;7(11):1426-1449. doi:10.15252/emmm.201404976

89. Bai L, Mao R, Wang J, et al. Erk1/2 promoted proliferation and inhibited apoptosis of human cervical cancer cells and regulated the expression of c-fos and c-Jun proteins. *Med Oncol*. 2015;32(3):1-7. doi:10.1007/s12032-015-0490-5

90. Shi YH, Tuokan T, Lin C, Chang H. Aquaporin 8 involvement in human cervical cancer SHa migration via the EGFR-Erk1/2 pathway. *Asian Pac J Cancer Prev*. 2014;15(15):6391-6395. doi: 10.7314/APJCP.2014.15.15.6391

91. Rashmi R, DeSelm C, Helms C, et al. Akt inhibitors promote cell death in cervical cancer through disruption of mTOR signaling and glucose uptake. *PLoS One*. 2014;9(4):e92948. doi:10.1371/journal.pone.0092948

92. Yung MM, Chan DW, Liu VW, Yau KM, Ngan HY. Activation of AMPK inhibits cervical cancer cell growth through AKT/FOXO3a/FOXO1 signaling cascade. *BMC Caner*. 2013;13(1):1-8. doi:10.1186/1471-2407-13-327

93. Schwarz JK, Payton JE, Rashmi R, et al. Pathway-specific analysis of gene expression data identifies the PI3K/Akt pathway as a novel therapeutic target in cervical cancer. *Clin Cancer Res*. 2012;18(5):1464-1471. doi: 10.1158/1078-0432.CCR-11-2485

94. Xia S, Zhao Y, Yu S, Zhang M. Activated PI3K/Akt/COX-2 pathway induces resistance to radiation in human cervical cancer HeLa cells. *Cancer Biother Radiopharm*. 2010;25(3):317-323. doi:10.1089/cbr.2009.0707

95. Noordhuis MG, Eijnsink JJ, Ten Hoor KA, et al. Expression of epidermal growth factor receptor (EGFR) and activated EGFR predict poor response to (chemo)radiation and survival in cervical cancer. *Clin Cancer Res*. 2009;15(23):7389-7397. doi: 10.1158/1078-0432.CCR-09-1149

96. Prusty BK, Das BC. Constitutive activation of transcription factor AP-1 in cervical cancer and suppression of human papillomavirus (HPV) transcription and AP-1 activity in HeLa cells by curcumin. *Int J Cancer*. 2005;113(6):951-960. doi:10.1002/ijc.20668

97. Tao P, Wen H, Yang B, Zhang A, Wu X, Li Q. miR-144 inhibits growth and metastasis of cervical cancer cells by targeting VEGFA and VEGFC. *Exp Ther Med*. 2018;15(1):562-568. doi:10.3892/etm.2017.5392

98. Chen B, Zhang C, Dong P, Guo Y, Mu N. Molecular regulation of cervical cancer growth and invasion by VEGFA. *Tumor Biol*. 2014;35(11):11587-11593. doi:10.1007/s13277-014-2463-2

99. Braicu EI, Gasimli K, Richter R, et al. Role of serum VEGFA, TIMP2, MMP2 and MMP9 in monitoring response to adjuvant radiochemotherapy in patients with primary cervical cancer—results of a companion protocol of the randomized NOGGO-AGO phase III clinical trial. *Anticancer Res*. 2014;34(1):385-391.

100. Zhu X, Er K, Mao C, et al. miR-203 suppresses tumor growth and angiogenesis by targeting VEGFA in cervical cancer. *Cell Physiol Biochem*. 2013;32(1):64-73. doi:10.1159/000350125

101. Chen A, Xu Y, Qiu S, et al. Ly6K promotes cervical cancer growth, invasion and migration through regulating VEGFA. *Int J Clin Exp Pathol*. 2016;9(11):4361-4367.

102. Chung S-H, Franceschi S, Lambert PF. Prevention and treatment of cervical cancer in mice using estrogen receptor antagonists. *Proc Natl Acad Sci U S A*. 2009;106(46):19467-19472. doi:10.1073/pnas.0911436106

103. Chung S-H, Wiedmeyer K, Shai A, Korach KS, Lambert PF. Requirement for estrogen receptor alpha in a mouse model for human papillomavirus-associated cervical cancer. *Cancer Res*. 2008;68(23):9928-9934. doi:10.1158/0008-5472.CAN-08-2051
105. Brake T, Lambert PF. Estrogen contributes to the onset, persistence, and malignant progression of cervical cancer in a human papillomavirus-transgenic mouse model. Proc Natl Acad Sci U S A. 2005;102(7):2490-2495. doi:10.1073/pnas.0409883102

106. Kang Y-H, Lee S-J. Role of p38 MAPK and JNK in enhanced cervical cancer cell killing by the combination of arsenic trioxide and ionizing radiation. Oncol Rep. 2008;20(3):637-643.

107. Kang Y-H, Lee S-J. The role of p38 MAPK and JNK in arsenic trioxide-induced mitochondrial cell death in human cervical cancer cells. J Cell Physiol. 2008;217(1):23-33. doi:10.1002/jcp.21470

108. Zhang S, Lin Z-N, Yang C-F, Shi X, Ong C-N, Shen H-M. Suppressed NF-kappaB and sustained JNK activation contribute to the sensitization effect of parthenolide to TNF-alpha-induced apoptosis in human cancer cells. Carcinogenesis. 2004;25(11):2191-2199. doi:10.1093/carcin/10542

109. Liu B, Fang M, Lu Y, Lu Y, Mills GB, Fan Z. Involvement of JNK-mediated pathway in EGF-mediated protection against paclitaxel-induced apoptosis in SiHa human cervical cancer cells. Br J Cancer. 2001;85(2):303-311. doi:10.1054/bjoc.2001.1910

110. Lin C-L, Lee C-H, Chen C-M, et al. Protodioscin induces apoptosis through ROS-mediated endoplasmic reticulum stress via the JNK/p38 activation pathways in human cervical cancer cells. Cell Physiol Biochem. 2018;46(1):322-334. doi:10.1159/000488433

111. Wu C-H, Ko J-L, Chen S-C, et al. Clinical implications of aldo-keto reductase family 1 member C3 and its relationship with lipocidin 2 in cancer of the uterine cervix. Gynecol Oncol. 2014;132(2):474-482. doi:10.1016/j.ygyno.2013.11.032

112. Wanichwatanadecha P, Sirisrimangkorn S, Kaewprag J, Ponglikitmongkol M. Transactivation activity of human papillomavirus type 16 E6* on aldo-keto reductase genes enhances chemoresistance in cervical cancer cells. J Gen Virol. 2012;93(Pt 5):1081-1092. doi:10.1099/vir.0.038265-0

113. Yue SJ, Liu J, Feng WW, et al. System pharmacology-based dissection of the synergistic mechanism of Huangqi and Huangqin for diabetes mellitus. Front Pharmacol. 2017;8(694):1-17. doi:10.3389/fphar.2017.00694

114. Kolch W, Halasz M, Granovskaya M, Kholodenko BN. The dynamic control of signal transduction networks in cancer cells. Nat Rev Cancer. 2015;15(9):515-527. doi:10.1038/nrc3983

115. Bossler F, Hoppe-Seyler K, Hoppe-Seyler F. PI3K/Akt/mTOR signaling regulates the virus/host cell crosstalk in HPV-positive cervical cancer cells. Int J Mol Sci. 2019;20(9):1-13. doi:10.3390/ijms20092188

116. Shi X, Wang J, Lei Y, Cong C, Tan D, Zhou X. Research progress on the PI3K/Akt signaling pathway in gynecological cancer (review). Mol Med Rep. 2019;19(6):4529-4535. doi:10.3892/mmr.2019.10121

117. Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X. Targeting PI3K in cancer: mechanisms and advances in clinical trials. Mol Cancer. 2019;18(1):1-28. doi:10.1186/s12943-019-0954-x

118. 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(7):505-515. doi:10.1038/nrc1647

119. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer. 2003;3(10):721-732. doi:10.1038/nrc1187

120. del Campo JM, Prat A, Gil-Moreno A, Pérez J, Parera M. Update on novel therapeutic agents for cervical cancer. Gynecol Oncol. 2008;110(3 Suppl 2):S72-S76. doi:10.1016/j.ygyno.2008.04.016

121. Ghittoni R, Accardi R, Chiocca S, Tommasino M. Role of human papillomaviruses in carcinogenesis. Esancermedicalsciences. 2015;9:526. doi:10.3332/ecancer.2015.526

122. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet. 2007;370(9590):890-907. doi:10.1016/S0140-6736(07)61416-0

123. Feng W, Xiao J, Zhang B, et al. Senescence and apoptosis in carcinogenesis of cervical squamous carcinoma. Mod Pathol. 2007;20(9):961-966. doi:10.1038/modpathol.3800927

124. Rinaldi S, Plummer M, Biessy C, et al. Endogenous sex steroids and risk of cervical carcinoma: results from the EPIC study. Cancer Epidemiol Biomarkers Prev. 2011;20(12):2532-2540. doi:10.1158/1055-9965.EPI-11-0753

125. Bicho MC, Pereira da Silva A, Matos A, Silva RM, Bicho MD. Sex steroid hormones influence the risk for cervical cancer: modulation by haptoglobin genetic polymorphism. Cancer Genet Cytogenet. 2009;191(2):85-89. doi:10.1016/j.cancergeneto.2009.02.005

126. Mezaache I, Panicia B, Nyinawabera A, Nuovo GJ. Enhanced expression of PD-L1 in cervical intraepithelial neoplasia and cervical cancers. Mod Pathol. 2015;28(12):1594-1602. doi:10.1038/modpathol.2015.108

127. Lipson EJ, Forde PM, Hammers H-J, Emens LA, Taube JM, Topalian SL. Antagonists of PD-L1 and PD-L1 in cancer treatment. Semin Oncol. 2015;42(4):587-600. doi:10.1053/j.seminoncol.2015.05.013

128. de Arellano AR, Lopez-Pulido EJ, Martinez-Neri PA, et al. Stat3 activation is required for the antiproliferative effects of prolactin in cervical cancer cells. Cancer Cell. 2015;15(1):1-8. doi:10.1186/s12935-015-0234-9

129. Ascencio-Cedillo R, López-Pulido EJ, Muñoz-Valle JF, et al. Prolactin and prolactin receptor expression in cervical intraepithelial neoplasia and cancer. Pathol Oncol Res. 2015;21(2):241-246. doi:10.1007/s12253-014-9814-6

130. López-Pulido EJ, Muñoz-Valle JF, Del Toro-Arreola S, et al. High expression of prolactin receptor is associated with cell survival in cervical cancer cells. Cancer Cell Int. 2013;13(1):103-109. doi:10.1186/1475-2867-13-103

131. Li M, He Y, Peng C, Xie X, Hu G. Erianin inhibits human cervical carcinoma cell proliferation and elevates apoptosis via the mitochondrial and p53 signaling pathways. Oncol Lett. 2018;15(6):9845-9851. doi:10.3892/ol.2018.8571
133. Farhan M, Wang H, Gaur U, Little PJ, Xu J, Zheng W. Foxo signaling pathways as therapeutic targets in cancer. Int J Biol Sci. 2017;13(7):815-827. doi:10.7150/ijbs.20052

134. Xiao S, Zhou Y, Yi W, et al. Fra-1 is downregulated in cervical cancer tissues and promotes cervical cancer cell apoptosis by p53 signaling pathway in vitro. Int J Onco. 2015;46(4):1677-1684. doi:10.3892/ijo.2015.2873

135. Vidya Priyadarsini R, Senthil Murugan R, Maitreyi S, Ramalingam K, Karunagaran D, Nagini S. The flavonoid quercetin induces cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells through p53 induction and NF-kB inhibition. Eur J Pharm. 2010;649(1-3):84-91. doi:10.1016/j.ejphar.2010.09.020

136. Fernandes JV, DE Medeiros Fernandes TAA, DE Azevedo JCV, et al. Link between chronic inflammation and human papillomavirus-induced carcinogenesis. review. Oncol Lett. 2015;9(3):1015-1026. doi:10.3892/ol.2015.2884

137. Deivendran S, Marzook KH, Radhakrishna Pillai M. The role of inflammation in cervical cancer. Adv Exp Med Biol. 2014;816:377-399. doi:10.1007/978-3-0348-0837-8_15

138. Parida S, Mandal M. Inflammation induced by human papillomavirus in cervical cancer and its implication in prevention. Eur J Cancer Prev. 2014;23(5):432-448. doi:10.1097/CEJ.000000000000023

139. Liu L, Yang X, Chen X, et al. Association between TNF-α polymorphisms and cervical cancer risk: a meta-analysis. Mol Biol Rep. 2012;39(3):2683-2688. doi:10.1007/s11033-011-1022-9

140. Boccardo E, Lepique AP, Villa LL. The role of inflammation in HPV carcinogenesis. Carcinogenesis. 2010;31(11):1905-1912. doi:10.1093/carcin/bgp176

141. Yang M, Wang M, Li X, et al. Wnt signaling in cervical cancer? J Cancer. 2018;9(7):1277-1286. doi:10.7150/jca.22005

142. Bahrami A, Hasanazadeh M, ShahidSales S, et al. Clinical significance and prognosis value of Wnt signaling pathway in cervical cancer. J Cell Biochem. 2017;118(10):3028-3033. doi:10.1002/jcb.25992

143. Epstein RJ. Vegf signaling inhibitors: more pro-apoptotic than anti-angiogenic. Cancer Metastasis Rev. 2007;26(3-4):443-452. doi:10.1007/s10689-007-9071-1

144. Wei L-H, Kuo M-L, Chen K-C, et al. Interleukin-6 promotes cervical tumor growth by VEGF-dependent angiogenesis via a STAT3 pathway. Oncogene. 2003;22(10):1517-1527. doi:10.1038/sj.onc.1206226

145. Tokumo K, Kodama J, Seki N, et al. Different angiogenic pathways in human cervical cancers. Gynecol Oncol. 1998;68(1):38-44. doi:10.1006/gyno.1997.4876

146. Montojo J, Zuberi K, Rodriguez H, Bader GD, Morris Q. GeneMANIA: fast gene network construction and function prediction for Cytoscape. F1000Res. 2014;3:153. doi:10.12688/f1000research.4572.1

147. Li Y, Hong J, Li H, et al. Genkwain nanosuspensions: a novel and potential antitumor drug in breast carcinoma therapy. Drug Deliv. 2017;24(1):1491-1500. doi:10.1080/10717544.2017.1384519

148. Reis FS, Barros I, Calhelha RC, et al. The methanolic extract of Cordyceps militaris (L.) link fruiting body shows antioxidant, antibacterial, antifungal and antihuman tumor cells properties. Food Chem Toxicol. 2013;62:91-98. doi:10.1016/j.fct.2013.08.033

149. Lee H-J, Kim K-H, Park J-K, Hwang E-H. Effects of Artemisia capillaris Thunberg on apoptosis in HeLa cells. J Nutr Health. 2008;41(1):22-30.

150. Li M. Invitroanti-respiratory syncytial virus effect of the extraction of Lonicera japonica Thunbs. J Trop Med. 2010;10(4):420-422.

151. Lin T-H, Hsu W-H, Tsai P-H, et al. Dietary flavonoids, luteolin and quercetin, inhibit invasion of cervical cancer by reduction of UBE2S through epithelial-mesenchymal transition signaling. Food Funct. 2017;8(4):1558-1568. doi:10.1039/C6FO00551A

152. Kashafi E, Moradzadeh M, Mohamadkhani A, Erfanian S. Cordyceps militaris (L.) link fruiting body shows antioxidant, antibacterial, antifungal and antihuman tumor cell lines properties. J Korean Soc Food Sci Nutr. 2001;30(5):921-927.

153. Lin T-H, Hsu W-H, Tsai P-H, et al. Dietary flavonoids, luteolin and quercetin, inhibit invasion of cervical cancer by reduction of UBE2S through epithelial-mesenchymal transition signaling. Food Funct. 2017;8(4):1558-1568. doi:10.1039/C6FO00551A

154. Kashafi E, Moradzadeh M, Mohamadkhani A, Erfanian S. Cordyceps militaris (L.) link fruiting body shows antioxidant, antibacterial, antifungal and antihuman tumor cell lines properties. J Korean Soc Food Sci Nutr. 2001;30(5):921-927.

155. Tu L-Y, Bai H-H, Cai J-Y, Deng S-P. The mechanism of kaempferol induced apoptosis and inhibited proliferation in human cervical cancer SiHa cell: from macro to nano. Scanning. 2016;38(6):644-653. doi:10.1016/sca.21312

156. Ham S, Kim KH, Kwon TH, et al. Luteolin induces intrinsic apoptosis via inhibition of E6/E7 oncoproteins and activation of extrinsic and intrinsic signaling pathways in HPV-18-associated cells. Oncol Rep. 2014;31(6):2683-2691. doi:10.3892/or.2014.3157

157. Fu J, Chen D, Zhao B, et al. Luteolin induces carcinoma cell apoptosis through binding Hsp90 to suppress constitutive activation of STAT3. PLoS One. 2012;7(11):e49194. doi:10.1371/journal.pone.0049194

158. Xie F, Lang Q, Zhou M, et al. The dietary flavonoid luteolin inhibits Aurora B kinase activity and blocks proliferation of cancer cells. Eur J Pharm Sci. 2012;46(5):388-396. doi:10.1016/j.ejps.2012.03.002

159. Hortinaka M, Yoshida T, Shiraishi T, et al. Luteolin induces apoptosis via death receptor 5 upregulation in human malignant tumor cells. Oncogene. 2005;24(48):7180-7189. doi:10.1038/sj.onc.1208874

160. Hortinaka M, Yoshida T, Shiraishi T, et al. The combination of TRAIL and luteolin enhances apoptosis in human cervical cancer HeLa cells. Biochem Biophys Res Commun. 2005;333(3):833-838. doi:10.1016/j.bbrc.2005.05.179

161. Lin C-W, Lai G-M, Chen K-C, et al. Rps12 increases the invasiveness in cervical cancer activated by e-myc and inhibited by the dietary flavonoids luteolin and quercetin. J Funct Foods. 2015;19:236-247. doi:10.1016/j.jff.2015.09.030
162. Kedhari Sundaram M, Raina R, Afroze N, et al. Quercetin modulates signaling pathways and induces apoptosis in cervical cancer cells. *Biosci Rep*. 2019;39(8):1-17. doi: 10.1042/BSR20190720

163. Kedhari Sundaram M, Hussain A, Haque S, Raina R, Afroze N. Quercetin modifies 5'CpG promoter methylation and reactivates various tumor suppressor genes by modulating epigenetic marks in human cervical cancer cells. *J Cell Biochem*. 2019;120(10):18357-18369. doi: 10.1002/jcb.29147

164. Clemente-Soto AF, Salas-Vidal E, Milan-Pacheco C, Sánchez-Carranza JN, Peralta-Zaragoza O, González-Maya L. Quercetin induces G2 phase arrest and apoptosis with the activation of p53 in an E6 expression-independent manner in HPV-positive human cervical cancer-derived cells. *Mol Med Rep*. 2019;19(3):2097-2106. doi: 10.3892/mmr.2019.9850

165. Ali A, Kim MJ, Kim MY, et al. Quercetin induces cell death in cervical cancer by reducing O-GlcNAcylation of adenosine monophosphate-activated protein kinase. *Anat Cell Biol*. 2018;51(4):274-283. doi: 10.5115/acb.2018.51.4.274

166. Wang Y, Zhang W, Lv Q, Zhang J, Zhu D. The critical role of quercetin in autophagy and apoptosis in HeLa cells. *Tumour Biol*. 2016;37(1):925-929. doi: 10.1007/s13277-015-3890-4

167. Bądzioł D, Jakubowicz-Gil J, Paduch R, Głowniak K, Gawron A. Combined treatment with quercetin and imperatorin as a potent strategy for killing HeLa and HEp-2 cells. *Mol Cell Biochem*. 2014;392(1-2):213-227. doi: 10.1007/s11010-014-2032-4

168. Danihelová M, Veverka M, Sturdík E, Jantová S. Antioxidant action and cytotoxicity on HeLa and NIH-3T3 cells of new quercetin derivatives. *Interdiscip Toxicol*. 2013;6(4):209-216. doi: 10.2478/itox-2013-0031

169. Bishayee K, Ghosh S, Mukherjee A, Sadhukhan R, Mondal J, Khuda-Bukhsh AR. Quercetin induces cytochrome-c release and ROS accumulation to promote apoptosis and arrest the cell cycle in G2/M, in cervical carcinoma: signal cascade and drug-DNA interaction. *Cell Prolif*. 2013;46(2):153-163. doi: 10.1111/cpr.12017

170. Chen AY, Chen YC. A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. *Food Chem*. 2013;138(4):2099-2107. doi: 10.1016/j.foodchem.2012.11.139

171. Hattori M, Mizuguchi H, Baba Y, et al. Quercetin inhibits transcriptional up-regulation of histamine H1 receptor via suppressing protein kinase C-8/extracellular signal-regulated kinase/poly(ADP-ribose) polymerase-1 signaling pathway in HeLa cells. *Int Immunopharmacol*. 2013;15(2):232-239. doi: 10.1016/j.intimp.2012.12.030

172. Jung JH, Lee JO, Kim JH, et al. Quercetin suppresses HeLa cell viability via AMPK-induced Hsp70 and EGFR down-regulation. *J Cell Physiol*. 2010;223(2):408-414. doi: 10.1002/jcp.22049

173. Zhang W, Zhang F. Effects of quercetin on proliferation, apoptosis, adhesion and migration, and invasion of HeLa cells. *Eur J Gynaecol Oncol*. 2009;30(1):60-64

174. Jakubowicz-Gil J, Paduch R, Piersiak T, Głowniak K, Gawron A, Kandefer-Szerszeń M. The effect of quercetin on pro-apoptotic activity of cisplatin in HeLa cells. *Biochem Pharmacol*. 2005;69(9):1343-1350. doi: 10.1016/j.bcp.2005.01.022

175. Wei J, Su H, Bi Y, Li J, Feng L, Sheng W. Anti-proliferative effect of isorhamnetin on HeLa cells through inducing G2/M cell cycle arrest. *Exp Ther Med*. 2018;15(4):3917-3923. doi: 10.3892/etm.2018.5892

176. Jin Y, Meng X, Qiu Z, Su Y, Yu P, Qu P. Anti-tumor and anti-metastatic roles of cordycepin, one bioactive compound of *Cordyceps militaris*. *Saudi J Biol Sci*. 2018;25(5):991-995. doi: 10.1016/j.jsbs.2018.05.016

177. Tania M, Shawon J, Saif K, et al. Cordycepin downregulates CDK-2 to interfere with cell cycle and increases apoptosis by generating ROS in cervical cancer cells: *in vitro* and *in silico* study. *Curr Cancer Drug Targets*. 2019;19(2):152-159. doi: 10.2174/1568009618666180905095356

178. Seong DB, Hong S, Muthusami S, Kim W-D, Yu J-R, Park W-Y. Cordycepin increases radiosensitivity in cervical cancer cells by overriding or prolonging radiation-induced G2/M arrest. *Eur J Pharmacol*. 2016;771:77-83. doi: 10.1016/j.ejphar.2015.12.022

179. Vanhoefer U, Harstrick A, Achterrath W, Cao S, Seeber S, Rustum YM. Irinotecan in the treatment of colorectal cancer: clinical overview. *J Clin Oncol*. 2001;19(5):1501-1518. doi: 10.1200/JCO.2001.19.5.1501