A Comprehensive Understanding of the Anticancer Mechanisms of FDY2004 Against Cervical Cancer Based on Network Pharmacology

Ho-Sung Lee¹², In-Hee Lee¹, Kyungrae Kang², Sang-In Park³, Minho Jung², Seung Gu Yang⁴, Tae-Wook Kwon², and Dae-Yeon Lee¹²

Abstract
Herbal drugs are continuously being developed and used as effective therapeutics for various cancers, such as cervical cancer (CC); however, their mechanisms of action at a systemic level have not been explored fully. To study such mechanisms, we conducted a network pharmacological investigation of the anti-CC mechanisms of FDY2004, an herbal drug consisting of Moutan Radicis Cortex, Persicae Semen, and Rhei Radix et Rhizoma. We found that FDY2004 inhibited the viability of human CC cells. By performing pharmacokinetic evaluation and network analysis of the phytochemical components of FDY2004, we identified 29 bioactive components and their 116 CC-associated pharmacological targets. Gene ontology enrichment analysis showed that the modulation of cellular functions, such as apoptosis, growth, proliferation, and survival, might be mediated through the FDY2004 targets. The therapeutic targets were also key components of CC-associated oncogenic and tumor-suppressive pathways, including PI3K-Akt, human papillomavirus infection, IL-17, MAPK, TNF, focal adhesion, and viral carcinogenesis pathways. In conclusion, our data present a comprehensive insight for the mechanisms of the anti-CC properties of FDY2004.

Keywords
herbal drug, network pharmacology, cervical cancer, anticancer agents, molecular mechanisms

Received: February 26th, 2021; Accepted: March 2nd, 2021.

Cervical cancer (CC), a malignant tumor that occurs owing to the abnormal control of the cellular behavior of cervical cells, is a highly incident gynecologic cancer possessing high mortality with more than 0.5 million cases and 0.3 million deaths per year worldwide.¹² Key molecular and pathway mechanisms for CC pathology include the dysregulation of multiple oncogenes and tumor suppressors, and their associated signaling and gene regulatory cascades.³⁴ Growing knowledge regarding the pathomechanisms of CC has advanced the design and development of chemotherapy, targeted therapy, and cancer immunotherapy, which are the most widely used CC therapies at present;⁵ however, they frequently cause resistance and side effects. Along with these concerns, substantial attention has been given to the development and usage of herbal drugs, the multiple component-multiple target polypharmacological agents with potent efficacy and safety, for cancer treatment.⁶⁻¹⁰ Herbal drugs elevate the clinical outcome and lower the toxicity of anticancer treatments, thereby improving the cancer-related symptoms and health status of cancer patients.¹¹

FDY2004 is an herbal drug consisting of Moutan Radicis Cortex (MRC), Persicae Semen (PS), and Rhei Radix et Rhizoma (RRR).¹² This herbal agent has demonstrated potent anticancer properties in breast and lung cancer cells.¹² However, its pharmacological activity against CC has not been examined.

Because herbal drugs show their therapeutic activities in a complex multiple phytochemical component-multiple targeted-manner, network pharmacology, a research concept that combines pharmacology, medicine, and network science and effectively facilitates the mechanistic investigation of polypharmacological drugs, is widely used to uncover the comprehensive mechanisms of herbal drugs.⁹⁻¹³⁻¹⁶ Network
pharmacology analysis identifies the major bioactive phytochemical components and therapeutic targets that are important for the pharmacological action of herbal drugs, integrates herbal drug-associated comprehensive data into diverse types of networks, and investigates pharmacological mechanisms of herbal drugs by analyzing their network properties.9,13-16 Using this network pharmacology methodology, we studied the therapeutic activity of FDY200417 in CC treatment.

Materials and Methods

Preparation of FDY2004

Dried raw plant materials of MRC (6.67 g), PS (6.67 g), and RRR (6.67 g) were purchased from Green Myeong-poom Pharm. (Namyangju, South Korea). Then, they were ground and mixed, and further added to 200 ml of distilled water and boiled for 120 minutes at 100 °C. After filtration with a 1-μm-pore filter (Hyundai Micro, Seoul, South Korea), the herbal extract was lyophilized at −80 °C. The lyophilized samples were kept at −20 °C and dissolved in distilled water for experimental use.

Cell Culture

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

Cell Viability Assay

The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA) assay was conducted to measure cell viability.17 Cells were seeded at 5.0 × 10⁴ cells per well in 48-well plates and treated with FDY2004 for 48 hours in 5% CO₂ at 37 °C. Cells were then incubated for 2 hours after adding 200 µL of MTT to the contents of each well. Afterwards, the MTT solution was removed and dimethyl sulfoxide was added to dissolve the formed formazan crystals. The absorbance was assessed using an Epoch 2 microplate reader (BioTek, Winooski, VT, USA) at 550 nm.

Determination of the Bioactive Phytochemical Components of FDY2004

The pharmacokinetic information (eg, oral bioavailability, drug-likeness, and Caco-2 permeability) of the phytochemical components of FDY2004 was investigated using the Traditional Chinese Medicine Systems Pharmacology 18, Traditional Chinese Medicine Integrated Database,18 Anticancer Herbs Database of Systems Pharmacology,19 and Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine20 databases. Then, bioactive phytochemical components were determined according to the following criteria, as previously described: oral bioavailability ≥30%, Caco-2 permeability ≥−0.4, and drug-likeness ≥0.18.14 Oral bioavailability refers to the quantitative proportion of the chemical components reaching the target sites (eg, tissues and organs) after oral administration; those with oral bioavailability ≥30% are expected to possess sufficient pharmacological absorption capability in the body.21,22 Drug-likeness is a parameter indicating the qualitative assessment of chemical components to determine their suitability for drug usage based on their molecular information and Tanimoto coefficients21,23; chemical components having drug-likeness ≥0.18 are considered to retain drug-like properties because the value of 0.18 is the average drug-likeness of available drugs.21,23 Caco-2 permeability is a coefficient for evaluating the intestinal permeability, flux rate, and ability to transport chemical components using human Caco-2 intestinal cells; generally, those with Caco-2 permeability ≥−0.4 are considered to exhibit suitable permeability in the intestinal epithelium.24,25

Targets of the Bioactive Phytochemical Components of FDY2004

The simplified molecular-input line-entry system (SMILES) notation of the phytochemical components of FDY2004 were extracted from the PubChem database,26 and imported into the following databases to obtain the human molecular targets: Similarity Ensemble Approach,27 SwissTargetPrediction,28 Search Tool for Interactions of Chemicals 5,29 and PharmMapper.30 The targets associated with initiation, development, and progression of CC were collected from the following databases using “Uterine Cervical Neoplasms” (Medical Subject Headings Unique ID: D002583) as the search keyword: Therapeutic Target Database,14 Comparative Toxicogenomics Database,31 DrugBank,32 Human Genome Epidemiology Navigator,33 DisGeNET,34 Online Mendelian Inheritance in Man,35 GeneCards,36 and Pharmacogenomics Knowledgebase.37

Construction of the Herbal Drug-Related Networks

In network pharmacology, a network consists of nodes (eg, herbal medicines, bioactive phytochemical components, targets, or pathways), and edges (or links) representing interactions between them.39 The number of edges connected to a node is called the degree.39 Comprehensive data regarding the relationship between the herbal medicines and their bioactive phytochemical components and their components and therapeutic targets were merged into an herbal medicine-bioactive phytochemical component-target (H-C-T) network. The herbal medicine-bioactive phytochemical component-target-pathway (H-C-T-P) network was generated by linking the targets in the H-C-T network with their enriched pathways. A protein-protein interaction (PPI) network was created using the paired interaction between the targets with a confidence score ≥0.9
from the STRING database. Nodes were considered as hubs if their degrees met the following criteria, ≥2 × average degree of all nodes in the PPI network, as previously described. The constructed H-C-T, H-C-T-P, and PPI networks were visualized using Cytoscape software.

**Survival Analysis**

We used Kaplan-Meier Plotter to perform the survival analysis of the FDY2004 targets.

**Functional Analysis of the FDY2004 Targets**

We used gProfiler and Kyoto Encyclopedia of Genes and Genomes for the gene ontology (GO) and pathway enrichment analysis of the FDY2004 targets, respectively. Functional interaction analysis was conducted using GeneMANIA.

**Molecular Docking Analysis**

Information on the 3-dimensional structures of the bioactive phytochemical components and targets of FDY2004 was surveyed from the PubChem database and the RCSB Protein Data Bank database, respectively. Next, the molecular docking scores between them were evaluated by importing their structural information into Autodock Vina. We considered the phytochemical component-target pairs with docking scores ≤ −5 to exhibit stable physicochemical binding affinities, as previously described.

**Results**

**Effects of FDY2004 Treatment on Cervical Cancer Cells**

To investigate the inhibitory effects of FDY2004 against CC, we treated HeLa cells with the herbal drug and observed their cell viability. The HeLa cells showed a significant reduction in their viability after FDY2004 treatment (Supplemental Figure S1), indicating the anticancer property of the herbal medicine against CC.

**Bioactive Phytochemical Components of FDY2004 and Their Targets**

Among the phytochemical components of FDY2004 investigated from herbal medicine-associated databases (Supplemental Table S1), we screened the bioactive components among those using the criteria mentioned earlier (see Materials and Methods). In addition, some phytochemical components that unsatisfied the criteria but are present in large quantities in the herbal medicines and possess effective therapeutic activity were also regarded as bioactive components. Consequently, we identified 35 bioactive components for FDY2004 (Supplemental Table S2). Thereafter, using various databases for the investigation of protein-chemical interactions and disease-associated genes and proteins, we obtained 212 targets (116 CC-associated and 96 non-CC-associated) for the 29 bioactive phytochemical components (Supplemental Table S3).

**Network-Perspective Analysis of the Mechanism of FDY2004**

The FDY2004-related detailed information was integrated into the H-C-T network with 148 nodes (3 herbal medicines, 29 bioactive phytochemical components, and 116 CC-related targets) and 271 links between them (Figure 1 and Supplemental Table S3). Quercetin and kaempferol were the bioactive components with the largest number of targets (Figure 1, Supplemental Table S3), suggesting their crucial pharmacological roles. Furthermore, 93.10% (27 out of 29) of the phytochemical components shared one or more target, and 77.59% (90 out of 116) of the CC-associated targets interacted with 2 or more herbal medicines (Figure 1), indicating the multiple component-multiple target effects of FDY2004.

To gain insight into the interactions between the CC-associated FDY2004 targets, we investigated the topological features of the PPI network (92 nodes and 231 links) in which the targets serve as nodes and their interactions as links (Figure 2). In the network analysis, we identified the high-degree hub nodes that have important biological functions and potential as key therapeutic targets (see Materials and Methods). As a result, the nodes AKT1, EGFR, ESR1, HSP90AA1, JUN, PTK2, TNF, TP53, and VEGFA were determined as hubs, indicating that they are the major targets for the therapeutic activities of FDY2004 against CC (Figure 2). Furthermore, the expression levels of these nodes correlated with the survival outcome of patients with CC (Figure 3), which indicates their clinical significance. In addition, GeneMANIA analysis indicated the biological mechanisms underlying the interaction between the hubs, including the physical interaction, co-expression, and genetic interaction (Supplemental Figure S2). Altogether, the results demonstrate the network-perspective characteristics underlying the pharmacological activity of FDY2004 for CC treatment.

**Investigation of Functional Enrichment of FDY2004 Networks**

To dissect the biological mechanisms of the anti-CC activity of FDY2004, we analyzed the GO terms enriched for its targets. We found that the modulation of cellular behaviors, such as apoptosis, growth, proliferation, and survival, may be mediated by the FDY2004 targets (Supplemental Figure S3), thus demonstrating the molecular mechanisms of FDY2004.

Aberrant activation and/or inactivation of various signaling pathways is the key mechanism of cancer pathology. By analyzing the pathway enrichment for the FDY2004 targets, we found that the targets were major components of the following CC-associated pathways: “Apoptosis,” “Cellular senescence,” “ErbB signaling pathway,” “Estrogen signaling pathway,” “Focal adhesion,” “HIF-1 signaling pathway,” “Human papillomavirus
infection,” “IL-17 signaling pathway,” “MAPK signaling pathway,” “NF-kappa B signaling pathway,” “p53 signaling pathway,” “Pathways in cancer,” “PD-L1 expression and PD-1 checkpoint pathway in cancer,” “PI3K-Akt signaling pathway,” “Platinum drug resistance,” “Prolactin signaling pathway,” “Ras signaling pathway,” “TNF signaling pathway,” “VEGF signaling pathway,” and “Viral carcinogenesis” (Figure 4 and Supplemental Figure S3).

The overall functional enrichment analysis suggests the pharmacological regulatory properties of FDY2004 for CC treatment at the molecular and pathway levels.

**Molecular Docking Analysis**

Using in silico molecular docking techniques, we examined the binding affinities of the chemical constituents and their CC-associated targets. The interactions between the bioactive phytochemical components and their hub targets showed molecular docking scores ≤ −5.0 (Figure 5), which suggests the potential binding capacities between them and further confirms the results of the network pharmacological analysis.

**Discussion**

Globally, CC is among the most incident, prevalent, and fatal malignant cancer affecting women. Herbal drugs are increasingly being considered as effective cancer therapeutics for their pharmacological efficacy and reduced toxicity. Using a network pharmacology-based methodology, we investigated the therapeutic activity of FDY2004 against CC. We found that FDY2004 exerted anticancer effects on CC cells. The overall analysis results identified 29 bioactive phytochemical components of FDY2004 that interact with 116 CC-related targets. GO enrichment investigation suggested that the targets may participate in the modulation of apoptosis, growth, proliferation, and survival. Diverse signaling associated with CC initiation and progression, including phosphoinositide 3-kinase (PI3K)-Akt, human papillomavirus (HPV) infection, interleukin (IL)−17, mitogen-activated protein kinase (MAPK), tumor necrosis factor (TNF), focal adhesion, and viral carcinogenesis pathways, may confer the polypharmacological anti-CC effects of FDY2004.

The hub targets of FDY2004 may serve as key regulators underlying the pathological processes of CC and have druggable potential. AKT (encoded by AKT1), c-Jun (encoded by JUN), and epidermal growth factor receptor (EGFR; encoded by EGFR) are implicated in the modulation of various cellular processes in CC cells, such as growth, proliferation, migration, invasion, metastasis, angiogenesis, survival, and apoptosis, and they are radio- and chemo-sensitizers of anticancer therapies for CC. The overexpression of Akt1 and EGFR is associated with the poor survival and clinical outcomes of patients with CC. The oncogene JUN is upregulated in the CC tumor tissues compared to normal tissues, and lower JUN expression is related to higher survival among patients with CC. The estrogen receptor α (encoded by ESR1) is associated with the tumorigenesis, development, and persistence of CC. Its expression level is downregulated in CC tissues, which is correlated with decreased survival rate among patients with CC. Heat shock protein 90-α (encoded by HSP90AA1) plays a role in the regulation of anti-tumor immunity, cancer cell growth, and epithelial-to-mesenchymal transition (EMT) of CC cells, and its higher expression and activity is seen in the CC
Focal adhesion kinase (encoded by \textit{PTK2}) suppresses apoptosis but promotes growth, proliferation, survival, EMT, tumorigenesis, migration, and invasion of CC cells and its expression is positively correlated with the progression, metastasis, angiogenesis, and the poor prognosis and clinical outcomes of patients with CC.\textsuperscript{79-86} The expression and activity of TNF-\(\alpha\) (encoded by \textit{TNF}) is associated with the apoptosis, chemosensitivity, DNA damage response, necrosis, proliferation, invasion, and EMT of CC cells, and its polymorphisms are further correlated with the risk of development, progression, and malignancy in patients with CC.\textsuperscript{87-100} The genetic or epigenetic malfunction of \textit{p53} (encoded by \textit{TP53}), a key tumor suppressor that coordinates the cell cycle process, proliferation, apoptosis, DNA damage repair, and metabolism, can drive CC pathogenesis; therefore, the restoration of its expression or functional activity has been suggested as a potential strategy for treating CC.\textsuperscript{101-103} In addition, elevated expression and polymorphisms of \textit{TP53} are commonly observed in CC tissues compared to normal cervical tissues, and correlated with the poor clinical outcome of patients with CC.\textsuperscript{104,105} Vascular endothelial growth factor-A (VEGF-A; encoded by \textit{VEGFA}) is involved in the proliferation, metastasis, angiogenesis, and growth processes of CC cells,\textsuperscript{106-110} and it is upregulated in CC tissues.\textsuperscript{109,111} Moreover, the complex interactions between these hub targets via various genetic and signaling mechanisms may modulate diverse CC-associated pathologic cellular processes, confer the pharmacological effects of anti-CC therapies, and contribute to the development of therapeutic resistance.\textsuperscript{60,85,112-121}
The FDY2004-targeted pathways are the key signaling mechanisms mediating CC pathology and its therapeutic strategies. Dysregulation of erythroblastic leukemia viral oncogene homologue (ErbB), focal adhesion, hypoxia-inducible factor 1 (HIF-1), MAPK, PI3K-Akt, and Ras pathways are important for CC initiation, development, and progression. Abnormal activity of the estrogen signaling pathway is one of the major drivers of CC, and high expression levels of its pathway components are related to the risk of CC. Viral infection with HPV is one of the most crucial contributors to CC carcinogenesis. The key components of the IL-17 pathway play a role in the carcinogenesis of CC with viral infection, and their activity functions as a prognostic indicator for patients with CC. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling modulates the growth, cell cycle, migration, invasion, survival, apoptosis, immunomicroenvironment, autophagy, and the pathomechanisms associated with the viral infection of CC cells. The p53 pathway is a pharmacological mediator of anticancer therapy-induced intervention of the survival, migration, invasion, and proliferation of CC cells. The upregulated activity of

Figure 3. Survival analysis of the cervical cancer-related targets of FDY2004. Kaplan-Meier curves for the survival of patients with cervical cancer having high or low expression levels of the indicated targets.

Figure 4. Herbal medicine-bioactive phytochemical component-target-pathway network of FDY2004. Green nodes, herbal medicines; red nodes, bioactive phytochemical components; blue nodes, targets; orange nodes, pathways.
programmed death-ligand 1 (PD-L1)/programmed cell death protein 1 (PD-1) is implicated in the decreased survival of patients with CC and targeting this pathway can elevate anti-tumor immunity.\(^{145,146}\) The hyperactivation of the anti-apoptotic prolactin pathway may enhance the survival and proliferative capacity of CC cells.\(^{147,148}\) The TNF pathway controls the pro-tumorigenic inflammatory processes of CC cells and is linked with HPV-associated CC progression.\(^{91,149-151}\) Dysregulated activation of the VEGF pathway promotes the metastasis and angiogenesis of tumors, which in turn promotes CC progression and advancement.\(^{152,153}\)

The bioactive phytochemical components of the herbal medicines comprising FDY2004 have been reported to exhibit pharmacological effects against CC, which supports the anti-CC activity of this herbal drug. Aloe-emodin inhibits cell cycle progression, proliferation, growth, and survival, but enhances the apoptosis and radiosensitivity of CC cells.\(^{154-156}\) The antitumor mechanisms of caffeic acid include the enhancement of apoptotic cell death, chemosensitivity, and cell cycle arrest and the blockage of the proliferation and EMT of CC cells; these pharmacological effects are exhibited via the modulation of transforming growth factor (TGF)-\(\beta\), HIF-1, p53, mitochondrial, and adenosine monophosphate-activated protein kinase (AMPK) pathways.\(^{157-161}\) Catechins possess anticancer potential that may cause growth-suppression and apoptosis of CC cells mediated by caspase activation and induction of oxidative stress.\(^{162,163}\) Chrysophansol, paconiflorin, paconol, and pentagalloylglucose can repress the survival and proliferation of CC cells while promoting cell cycle arrest and apoptosis.\(^{164-167}\) Emodin suppresses the migration, invasion, and proliferation, and causes the oxidative stress, DNA damage, and apoptosis of CC cells by modulating the PI3K-Akt, HIF-1, VEGF, TGF-\(\beta\), mitochondrial, and death receptor signaling pathways.\(^{168-171}\) Gallic acid is an anticancer compound that modulates the activities of oncogenic ErbB, PI3K-Akt, MAPK, p53, caspase, and HPV signaling, which leads to the augmentation of apoptosis and necrosis and suppression of growth, proliferation, angiogenesis, and chemo-resistance.\(^{172-174}\) The pro-apoptotic and anti-proliferative role of hederagenin is mediated by the modulation of signal transducer and activator of transcription (STAT)–3 pathway.\(^{175}\) Kaempferol regulates the activities of PI3K-Akt and telomerase reverse transcriptase (hTERT) pathways to promote apoptotic cell death and suppress the growth and survival of CC cells.\(^{176}\) Mairin (betulinic acid) stimulates antitumor processes such as apoptosis, oxidative stress, and antiproliferation of CC cells that are conferred through the HIF, VEGF, TNF, PI3K-Akt, caspase, reactive oxygen species (ROS)-mediated mitochondrial, and endoplasmic reticulum (ER) pathways.\(^{177,179}\) Rhein-induced growth repression and apoptosis of CC cells may occur through the caspase and calcium pathways.\(^{180}\) The anticancer component quercetin inhibits cell cycle progression, proliferation, survival, viability, metastasis, migration, invasion, and EMT of CC cells by modulating diverse signaling mechanisms, including HPV, p53, ER stress, ROS, PI3K-Akt, mitochondrial, DNA damage.
response, epigenetic, p53, caspase, MAPK, HIF-1, Wnt, and NF-κB pathways. β-Sitosterol controls the expression and activity of HPV E6 and p53, which induces the apoptosis and arrests the growth of CC cells.

Limitations of the current study include the lack of experiments that evaluate the anti-CC effects and toxicity of combined treatment of FDY2004 with other anticancer agents involved in chemotherapy, targeted therapy, and cancer immunotherapy. Further investigations are needed to expand the therapeutic application of herbal drugs as anticancer therapies.

In summary, the overall network pharmacology-based analysis demonstrated the anti-CC properties of FDY2004. Our study offers an in-depth and systematic understanding of the polypharmacological mechanisms of herbal drugs, which will contribute to the design and development of improved anticancer herbal agents.

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. Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence involved in chemotherapy, targeted therapy, and cancer immunotherapy. Further investigations are needed to expand the therapeutic application of herbal drugs as anticancer therapies.

In summary, the overall network pharmacology-based analysis demonstrated the anti-CC properties of FDY2004. Our study offers an in-depth and systematic understanding of the polypharmacological mechanisms of herbal drugs, which will contribute to the design and development of improved anticancer herbal agents.

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. Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health. 2020;8(2):e191-e203. doi:10.1016/S2214-109X(19)30482-6
3. Manzo-Merino J, Contreras-Paredes A, Vázquez-Ulloa E, Rocha-Zavaleta L, 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
4. Zhang L, Wu J, Ling MT, Zhao L, Zhao K-N. The role of the PI3K/Akt/mTOR signalling pathway in human cancers induced by infection with human papillomaviruses. Mol Cancer. 2015;14:87 doi:10.1186/s12943-015-0361-x
5. Kumar L, 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.crpcancer.2018.01.016
6. Menederes G, Black J, Schwab CL, Santin AD. Immunotherapy and targeted therapy for cervical cancer: an update. Expert Rev Anticancer Ther. 2016;16(1):83-98. doi:10.1586/14737140.2016.1121108
7. 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
8. Ohnishi S, Takeda H. Herbal medicines for the treatment of cancer chemotherapy-induced side effects. Front Pharmacol. 2015;6(Suppl. 5):14 doi:10.3389/fphar.2015.00014
9. 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. Pharmazie. 2016;111:290-302. doi:10.1016/j.phrs.2016.06.018
10. Yin S-Y, Wei W-C, Jian F-Y, Yang N-S. Therapeutic applications of herbal medicines for cancer patients. Evid Based Complement Alternat Med. 2013;2013:1-15. doi:10.1155/2013/30246
11. SY O, Kim MS, Joo JC, Song YS. Efficacy of herbal medicine as an adjunctive therapy of chemotherapy for cervical cancer: a systematic review and meta-analysis. J Physiol Pathol Korean Med. 2020;34(5):255-262.
12. Lee I-H, Lee H-S, Kang K, et al. Influence of decoction duration of FDY2004 on its physicochemical components and antioxidant and antiproliferative activities. Nat Prod Commun. 2020;15(10) doi:10.1177/1934578X20968437
13. Lee W-Y, Lee C-Y, Kim Y-S, Kim C-E. The methodological trends of traditional herbal medicine employing network pharmacology. Biomolecules. 2019;9(8):362-15. doi:10.3390/biom9080362
14. Lee H-S, Lee I-H, Park S-I, Lee D-Y. Network Pharmacology-Based investigation of the system-level molecular mechanisms of the hematopoietic activity of Samul-Tang, a traditional Korean herbal formula. Evid Based Complement Alternat Med. 2020;2020:1-17. doi:10.1155/2020/9048089
15. Zhang SQ, Xu HB, Zhang SJ, Li XY. Identification of the active compounds and significant pathways of Artemisia annua in the treatment of non-small cell lung carcinoma based on network pharmacology. Med Sci Monit. 2020;26:e923624. doi:10.12659/msm.923624
16. Lee H-S, Lee I-H, Kang K, Park S-I, Kwon T-W, Lee D-Y. Investigation of the molecular mechanisms underlying the analgesic effect of Jakyak-Gamcho decoction: a network pharmacology study. Evid Based Complement Alternat Med. 2020;2020:1-17. doi:10.1155/2020/6628641
17. Lee D-Y, Lee I-H. FDY003 inhibits colon cancer in a COLO205 xenograft mouse model by decreasing oxidative stress. Pharmaceutic. 2019;15(65):675-681. doi:10.4103/pm.pm_650_18
18. Huang L, Xie D, Yu Y, et al. TCMD 2.0: a comprehensive resource for TCM. Nucleic Acids Res. 2018;46(D1):D1117-D1120. doi:10.1093/nar/gkx1028
19. Tao W, Li B, Gao S, et al. CancerHSP: anticancer herbs database of systems pharmacology. Sci Rep. 2015;5:11481. doi:10.1038/srep11481
20. Liu Z, Guo F, Wang Y, et al. BATMAN-TCM: a bioinformatics analysis tool for molecular mechanisms of traditional Chinese medicine. Sci Rep. 2016;6:21146. doi:10.1038/srep21146
21. Ru J, Li P, Wang J, et al. TC MSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform. 2014;6:13. doi:10.1186/1758-2946-6-13

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

23. Lee AY, Park W, Kang T-W, Cha MH, Chun JM. Network pharmacology-based prediction of active compounds and molecular targets in Yijin-Tang acting on hyperlipidemia and atherosclerosis. J Ethnopharmacol. 2018;221:151-159. doi:10.1016/j.jep.2018.04.027

24. Li Y, Zhang J, Zhang L, et al. Systems pharmacology to decipher the combinational anti-migraine effects of Tianshu formula. J Ethnopharmacol. 2015;174:45-56. doi:10.1016/j.jep.2015.07.043

25. Zhang J, Li Y, Chen X, Pan Y, Zhang S, Wang Y. Systems pharmacology dissection of multi-scale mechanisms of action in stroke treatment and prevention. PLoS One. 2014;9(8):e102506. doi:10.1371/journal.pone.0102506

26. Kim S, Chen J, Cheng T, et al. PubChem 2019 update: improved access to chemical data. Nucleic Acids Res. 2019;47(D1):D1102-D1109. doi:10.1093/nar/gky1033

27. Keiser MJ, Roth BL, Armbuster BN, Ernsberger P, Irwin JJ, Shoichet BK. Relating protein pharmacology by ligand chemistry. Nucleic Acids Res. 2007;25(2):197-206. doi:10.1038/nbt1284

28. Daina A, Michelin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019;47(D1):W357-W364. doi:10.1093/nar/gkz382

29. Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Irwin JJ, Shoichet BK. Augmenting protein-chemical interaction networks with tissue and affinity data. Nucleic Acids Res. 2016;44(D1):D80-D834. doi:10.1093/nar/gkw1277

30. Wang X, Shen Y, Wang S, et al. PharmMapper 2017 update: improved access to chemical data. Nucleic Acids Res. 2018;46(D1):D607-D613. doi:10.1093/nar/gky1131

31. 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/gkp1014

32. 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

33. 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

34. Yu W, Gwinn M, Clyne M, Yesupriya A, Khoury MJ. A navigator for human genome epidemiology. Nat Genet. 2008;40(2):124-125. doi:10.1038/ng0208-124

35. Piñero J, Bravo Alex, Queralt-Rosinach 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

36. 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

37. Safran M, Dalah I, Alexander J, et al. GeneCards version 3: the human gene integrator. Database. 2010;2010:baq020. doi:10.1093/database/baq020

38. 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

39. Babási A-, Olvai ZN. Network biology: understanding the cell's functional organization. Nat Rev Genet. 2004;5(2):101-113. doi:10.1038/nrg1272

40. Szklarczyk D, Gable AL, Lyon D, et al. String v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019;47(D1):D607-D613. doi:10.1093/nar/gky1131

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

42. 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 Xiaoxintong capsule in ischemic stroke treatment. Evid Based Complement Alternat Med. 2019;2019:1056708

43. Shannon Pet al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-2504. doi:10.1101/gr.1239303

44. Nagy Ádám, Lánczyk A, Menyhárt O, Györgyi B. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. Sci Rep. 2018;8(1):9227. doi:10.1038/s41598-018-27521-y

45. Raudvere U, Kolberg I, Kuzmin I, et al. gProfiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res. 2019;47(W1):W191-W198. doi:10.1093/nar/gkw369

46. Kancheisa M, Goto S. Kegg kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27-30. doi:10.1093/nar/28.1.27

47. 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

48. Burley SK, Berman HM, Bhikadiya C, et al. The rcsb protein data bank: biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. Nucleic Acids Res. 2019;47(D1):D464-D474. doi:10.1093/nar/gky1004

49. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-461. doi:10.1002/jcc.21334

50. 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.ejim.2020.101139

51. 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

52. Huang J, Cheung F, Tan H-Y, et al. Identification of the active compounds and significant pathways of yinchenhao decoction based on network pharmacology. *Med Mol Rep.* 2017;16(4):4583-4592. doi: 10.3892/mmr.2017.7149

53. Yue S-J, Xin L-T, Fan Y-C, et al. Herb pair Danggui-Honghua: mechanisms underlying blood stasis syndrome by system pharmacology approach. *Sci Rep.* 2017;7:40318. doi: 10.1038/srep40318

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

55. Jeong H, Mason SP, Barabási AL, Oltvai ZN. Lethality and sensitivity in squamous cervical cancer. *J Pharmacol.* 2016;791:297-307. doi: 10.1016/j.ejphar.2016.09.007

56. 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/nrc3989

57. Liu C, Ding L, Bai L, et al. Folate receptor alpha is associated with cervical carcinogenesis and regulates cervical cancer cell 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

58. Lu Z, Chen H, Zheng X-M, Chen M-L. 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.apjjm.2017.06.005

59. 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/jc.16607

60. 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-κB signaling. *Eur J Pharmacol.* 2016;791:297-307. doi: 10.1016/j.ejphar.2016.09.007

61. 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

62. 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

63. 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):57. doi: 10.1007/s12032-015-0490-5

64. Shi Y-H, 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

65. 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

66. Yung MMH, Chan DW, Liu VWS, Yao K-M, Ngan HY-S. Activation of AMPK inhibits cervical cancer cell growth through AKT/FOXO3a/FOXM1 signaling cascade. *BMC Cancer.* 2013;3:327. doi: 10.1186/1471-2407-13-327

67. 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

68. 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

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

70. 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

71. Kurnia IIN, Siregar B, Soetopo S, et al. Correlation between Akt and p53 protein expression and chemoradiotherapy response in cervical cancer patients. *Hayati.* 2014;21(4):173-179. doi: 10.4308/hj.21.4.173

72. Lai Y, Zhou B, Tan Q, Xu J, Wan T, Zhang L. LINCO0116 enhances cervical cancer tumorigenesis through miR-106a/c-Jun pathway. *J Cell Biochem.* 2020;121(3):2247-2257. doi: 10.1002/jcb.29447

73. Chung S-H, Franceschi S, Lambert PF, Estrogen LPE. Estrogen and ERalpha: culprits in cervical cancer? *Trends Endocrinol Metab.* 2010;21(8):504-511. doi: 10.1016/j.tem.2010.03.005

74. 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

75. 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.040983102

76. Dai F, Chen G, Wang Y, et al. Identification of candidate biomarkers correlated with the diagnosis and prognosis of cervical cancer via integrated bioinformatics analysis. *Onco Targets Ther.* 2019;12:4517-4532. doi: 10.2147/OTT.S199615

77. Song K-H, Oh SJ, Kim S, et al. Hsp90 inhibition promotes anti-tumor immunity by reversing multi-modal resistance and stem-like property of immune-refractory tumors. *Nat Commun.* 2020;11(1):562. doi: 10.1038/s41467-019-14259-y

78. Xu D, Dong P, Xiong Y, et al. MicroRNA-361-Mediated inhibition of Hsp90 expression and EMT in cervical cancer
is counteracted by oncogenic lncRNA NEAT1. *Cells*. 2020;9(3):1-20. doi:10.3390/cells9030032

79. Chen H, Suo K, Cheng Y, Zheng B, Xu L. Vascular endothelial growth factor C enhances cervical cancer migration and invasion via activation of focal adhesion kinase. *Gynecol Endocrinol*. 2013;29(1):20-24. doi:10.3109/09513590.2012.705387

80. Chen H, Wang D, Liu Y. SASH1 inhibits cervical cancer cell proliferation and invasion by suppressing the FAK pathway. *Med Mol Rep*. 2016;13(4):3613-3618. doi:10.3892/mmr.2016.4946

81. Chen Y, Hu X, Yang S. Clinical significance of focal adhesion kinase (FAK) in cervical cancer progression and metastasis. *Int J Clin Exp Pathol*. 2020;13(10):2586-2592.

82. Du Q, Wang W, Liu T, et al. High expression of integrin α3 predicts poor prognosis and promotes tumor metastasis and angiogenesis by activating the c-Src/Extracellular signal-regulated protein Kinase/Focal adhesion kinase signaling pathway in cervical cancer. *Front Oncol*. 2020;10:36. doi:10.3389/fonc.2020.00036

83. Gabriel B, zur Hausen A, Stickeler E, et al. Weak expression of focal adhesion kinase (pp125FAK) in patients with cervical cancer is associated with poor disease outcome. *Clin Cancer Res*. 2006;12(8):2476-2483. doi:10.1158/1078-0432.CCR-05-1867

84. Xu F, Zhang J, Hu G, Liu L, Liang W, Hypoxia. Hypoxia and TGF-β1 induced PLOD2 expression improve the migration and invasion of cervical cancer cells by promoting epithelial-to-mesenchymal transition (EMT) and focal adhesion formation. *Cancer Cell Int*. 2017;17:54. doi:10.1186/s12935-017-0420-z

85. Xu J, Zhu W, Chen L, Liu L. MicroRNA-433 inhibits cell growth and induces apoptosis in human cervical cancer through PI3K/Akt signaling by targeting FAK. *Oncol Rep*. 2018;40(6):3469-3478. doi:10.3892/or.2018.6718

86. Zhou Y, Shu C, Huang Y. Fibronectin promotes cervical cancer tumorigenesis through activating FAK signaling pathway. *J Cell Biochem*. 2019;10988-10997. doi:10.1002/jcb.28282

87. Barbisan G, Pérez LO, Contreras A, Golijow CD. TNF-α and IL-10 promoter polymorphisms, HPV infection, and cervical cancer risk. *Clin Exp Pathol*. 2020;13(10):2586-2592.

88. Jin Y, Qiu S, Shao N, Zheng J. Fucoxanthin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically promotes apoptosis of human cervical cancer cells by targeting PI3K/Akt/NF-κB signaling pathway. *Med Sci Monit*. 2018;24:11-18. doi:10.12659/MSM.905360

89. Li L, Liu J, Liu C, Lu X. The correlation between TNF-α-308 gene polymorphism and susceptibility to cervical cancer. *Oncol Lett*. 2018;15(5):7163-7167. doi:10.3892/ol.2018.8246

90. Li X, Yin G, Li J, et al. The correlation between TNF-α promoter gene polymorphism and genetic susceptibility to cervical cancer. *Technol Cancer Res Treat*. 2018;17:1533033818782797. doi:10.1177/1533033818782793

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

92. Lu JN, Lee WS, Yun JW, et al. Anthocyanins from Vitis coignetiae Pulliat inhibit cancer invasion and epithelial-mesenchymal transition, but these effects can be attenuated by tumor necrosis factor in human uterine cervical cancer HeLa cells. *Evid Based Complement Alternat Med*. 2013;2013:503043. doi:10.1155/2013/503043

93. Managit C, Sakurai H, Saiki I. Ethanolic extract of *Thevetia peruviana* flowers enhances TNF-α and TRAIL-induced apoptosis of human cervical cancer cells via intrinsic and extrinsic pathways. *Oncol Lett*. 2017;13(4):2791-2798. doi:10.3892/ol.2017.5748

94. Pan F, Tian J, Ji C-S, et al. Association of TNF-α-308 and -238 polymorphisms with risk of cervical cancer: a meta-analysis. *Asian Pac J Cancer Prev*. 2012;13(11):5777-5783. doi:10.7314/APJCP.2012.13.11.5777

95. Roszak A, Misztal M, Sowińska A, Jagodziński PP. TNF-α -308 G/A as a risk marker of cervical cancer progression in the Polish population. *Med Diagn Thor*. 2015;19(1):53-57. doi:10.1007/s40291-015-0130-y

96. Suk K, Chang I, Kim YH, et al. Interferon gamma (IFNgamma) and tumor necrosis factor alpha synergism in ME-180 cervical cancer cell apoptosis and necrosis. IFNgamma inhibits cytoprotective NF-κappa B through STAT1/IRF-1 pathways. *J Biol Chem*. 2001;276(16):13153-13159. doi:10.1074/jbc.M007646200

97. Sun X, Cui M, Wang D, Guo B, Zhang L. Tumor necrosis factor-related apoptosis inducing ligand overexpression and taxol treatment suppresses the growth of cervical cancer cells *in vitro* and *in vivo*. *Oncol Lett*. 2018;15(4):5744-5750. doi:10.3892/ol.2018.8071

98. Wang Y, Yang J, Huang J, Tian Z. Tumor necrosis factor-α polymorphisms and cervical cancer: evidence from a meta-analysis. *Gynecol Obstet Invest*. 2020;85(2):153-158. doi:10.1159/000502955

99. Xu L, Xu Q, Li X, Zhang X. Micromma-21 regulates the proliferation and apoptosis of cervical cancer cells via tumor necrosis factor-α. *Med Mol Rep*. 2017;46(4):4659-4663. doi:10.3892/mmr.2017.7143

100. Zhao Q, Wang W, Cui J. Melatonin enhances TNF-α-mediated apoptosis of cervical cancer HeLa cells death via suppressing CaMKII/Parkin/mitophagy axis. *Cancer Cell Int*. 2019;19:58. doi:10.1186/s12935-019-0777-2

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

102. Hietanen S, Lain S, Krausz E, Blattner C, Lane DP. Activation of p53 in cervical carcinoma cells by small molecules. *Front Oncol*. 2020;10:36. doi:10.3389/fonc.2020.00036

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

104. Klug SJ, Ressing M, Koening J, et al. Tp53 eodon 72 polymorphism and cervical cancer: a pooled analysis of individual data from 49 studies. *Lancet Oncol*. 2009;10(8):772-784. doi:10.1016/S1470-2045(09)70187-1
105. Lin J, Lu J, Wang C, Xue X. The prognostic values of the expression of vimentin, TP53, and podoplanin in patients with cervical cancer. Cancer Cell Int. 2017;17:80. doi:10.1186/s12935-017-0450-6

106. 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

107. Chen B, Zhang C, Dong P, Guo Y, Mu N. Molecular regulation of cervical cancer growth and invasion by VEGFA. Tumour Biol. 2014;35(11):11587-11593. doi:10.1016/j.tumres.2014.06.013

108. 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.

109. Zhu X, Er K, Mao C, et al. miR-203 suppresses tumor growth of cervical cells by a pentacyclic triterpenediol from Boswellia serrata. Phytomedicine. 2019.108957. doi:10.1016/j.phymed.2019.108957

110. Chen B, Zhang C, Dong P, Guo Y, Mu N. Molecular regulation of cervical cancer growth and invasion by VEGFA. Tumour Biol. 2014;35(11):11587-11593. doi:10.1016/j.tumres.2014.06.013

111. Guo J, Chen M, Ai G, Mao W, Li H, Zhou J. Hsa_circ_0023404 enhances cervical cancer metastasis and chemoresistance through VEGFA and autophagy signaling by sponging miR-5047. Biomed Pharmacother. 2019;115:108957. doi:10.1016/j.biopha.2019.108957

112. Bhushan S, Malik F, Kumar A, et al. Activation of p53/p21/WAF1, MIB-1, EGFR, HER2, and Bel-2 and clinical outcomes after curative chemoradiation therapy in squamous cell cervical cancer. Int J Radiat Oncol Biol Phys. 2009;74(4):1165-1172. doi:10.1016/j.ijrobp.2008.09.005

113. 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/ijms20192188

114. Chen A, Xu Y, Qiu S, et al. Ly6K promotes cervical cancer growth, invasion and migration through regulating VEGFA. Mol Cancer. 2018;17:80. doi:10.1186/s12935-017-0450-6

115. Chen Y, Yang S, Yang C, et al. Metformin induces apoptosis and epithelial-mesenchymal transition of cervical cancer via inactivation of the PI3K/AKT-mediated MDM2/p53 axis. Life Sci. 2020;259:118277. doi:10.1016/j.lfs.2020.118277

116. de Almeida VH, de Melo AC, Meira DD, et al. Radiotherapy and autophagy in gynecological cancer cell lines: a review. Braz J Med Biol Res. 2018;51(12):1141-1149. doi:10.1590/1414-431x20176822

117. Hakimee H, Hutamekalin P, Tanasswet S, Chonpathomphikulert P, Tipmanee V, Sukketsiri W. Metformin inhibit cervical cancer migration by suppressing the FAK/Akt signaling pathway. Asian Pac J Cancer Prev. 2019;20(12):3539-3545. doi:10.31557/apjcp.2019.20.12.3539

118. Hu L, Wang Y, Chen Z, et al. Hsp90 inhibitor SNX-2112 enhances TRAIL-induced apoptosis of human cervical cancer cells via the ROS-mediated JNK-p53-Autophagy-DR5 pathway. Oxid Med Cell Longev. 2019;2019:1-26. doi:10.1155/2019/9675450

119. Liu K, Xue B, Bai G, Zhang W. F-Box protein FBXO31 modulates apoptosis and epithelial-mesenchymal transition of cervical cancer via inactivation of the PI3K/AKT-mediated MDM2/p53 axis. Life Sci. 2020;259:118277. doi:10.1016/j.lfs.2020.118277

120. Muñoz JP, Carrillo-Beltrán D, Acelo-Aguilera V, et al. Tobacco exposure enhances human papillomavirus 16 oncogene expression via EGFR/PI3K/Akt/c-Jun signaling pathway in cervical cancer cells. Front Microbiol. 2018;9:3022. doi:10.3389/fmicb.2018.03022

121. Yamashita H, Murakami N, Asari T, Okuma K, Ohtomo K, Nakagawa K. Correlation among six biologic factors (p53, p21(WAF1), MIB-1, EGFR, HER2, and Bel-2) and clinical outcomes after curative chemoradiation therapy in squamous cell cervical cancer. Int J Radiat Oncol Biol Phys. 2009;74(4):1165-1172. doi:10.1016/j.ijrobp.2008.09.005

122. 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

123. 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(12):1-26. doi:10.1186/s12943-019-0954-x

124. Rinaldi S, Plummer M, Biessy C, et al. Endogenous sex steroids modulate apoptosis and epithelial-mesenchymal transition of cervical cancer via inactivation of the PI3K/AKT- mediated MDM2/p53 axis. Cancer Cell Int. 2018;18(1):26. doi:10.1186/s12943-019-0954-x

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

126. 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

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

128. 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):S72-S76. doi:10.1016/j.ygyno.2008.04.016

129. 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

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

131. Punt S, Fleuren GJ, Kruikou E, et al. Angels and demons: Th17 cells represent a beneficial response, while neutrophil IL-17 is associated with poor prognosis in squamous cervical cancer. Oncoimmunology. 2015;4(1):e984539. doi:10.4161/2162402X.2014.984539

132. Punt S, van Vliet ME, Spanns VM, et al. FoxP3(+) and IL-17(+) cells are correlated with improved prognosis in cervical cancer. Cancer Epidemiol Biomarkers Prev. 2019;28(5):1172-1181. doi:10.1158/1055-9965.EPI-18-0453
adenocarcinoma. Cancer Immunol Immunother. 2015;64(6):745-753. doi:10.1007/s00262-015-1678-4

132. Sun LX, Wang XB, Huang XJ. Association analysis of rs2275913G>A and rs763780T>C interleukin 17 polymorphisms in Chinese women with cervical cancer. Genet Mol Res. 2015;14(4):13612-1367. doi:10.4238/2015.October.22.28

133. Tartour E, Fossiez F, Joyceu I, et al. Interleukin 17, a T-cell-derived cytokine, promotes tumorigenicity of human cervical tumors in nude mice. Cancer Res. 1999;59(15):3698-3704.

134. Xue J, Wang Y, Chen C, Zhu X, Zhu H, Hu Y. Effects of Th17 cells and IL-17 on the progression of cervical carcinogenesis with high-risk human papillomavirus infection. Cancer Med. 2018;7(2):297-306. doi:10.1002/cam4.1279

135. Cai H, Yan L, Liu N, Xu M, Cai H. Ifi16 promotes cervical cancer progression by upregulating PD-L1 in immunomicroenvironment by STING-TBK1-NF-kB pathway. Biomed Pharmacother. 2020;123:109790. doi:10.1016/j.biopha.2019.109790

136. Ou J, Meng F, Liu J, Li D, Cao H, Sun B. Ovatodiolide exerts anticancer effects on human cervical cancer cells via mitotic catastrophe, apoptosis and inhibition of NF-kB pathway. J Biomed. 2020;25(1):87-92.

137. Vandermark ER, Deluca KA, Gardner CR, et al. Human papillomavirus type 16 E6 and E7 proteins alter NF-kB in cultured cervical epithelial cells and inhibition of NF-kB promotes cell growth and immortalization. Virology. 2012;425(1):53-60. doi:10.1016/j.virol.2011.12.023

138. Yu L, Sun Y, Su J, Li X. Bismarcanine exerts anticancer effects on human cervical cancer cells by inhibition of growth, migration and invasion via suppression of NF-kB signalling pathway. J Biomed. 2020;25(1):93-98.

139. Zhang L, Chinnathambi A, Alharbi SA, Veeraraghavan VP, Mohan SK, Zhang G. Pumicalgin promotes the apoptosis in human cervical cancer (ME-180) cells through mitochondrial pathway and by inhibiting the NF-kB signalling pathway. Saudi J Biol Sci. 2020;27(4):1100-1106. doi:10.1016/j.sjbs.2020.02.015

140. Zhang Y, Li G, Ji C. Inhibition of human cervical cancer tissues and promotes cervical cancer cell apoptosis by p53 signaling pathway in vitro. Int J Oncol. 2015;46(4):1677-1684. doi:10.3892/ijo.2015.2873

141. Zhang Y, Zhao Y, Ran Y, Guo J, Cui H, Liu S. Alantolactone exhibits selective antitumor effects in HeLa human cervical cancer cells by inhibiting cell migration and invasion, G2/M cell cycle arrest, mitochondrial mediated apoptosis and targeting NF-kB signalling pathway. J Biomed. 2019;24(6):2310-2315.

142. Li L, Qiu R-L, Lin Y, et al. Resveratrol suppresses 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

143. 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 Oncol. 2015;46(4):1677-1684. doi:10.3892/ijo.2015.2873
apoptosis in human cervical cancer cells via the mitochondrial pathways. *Onco Lett.* 2018;15(5):7397-7402. doi:10.3892/ol.2018.8256

158. Tyszka-Czochara M, Bukowska-Strakova K, Kocemba-Pilarczyk K, Majka M. Caffeic acid targets AMPK signaling and regulates tricarboxylic acid cycle anaplerosis while metformin downregulates HIF-1α-Induced glycolytic enzymes in human cervical squamous cell carcinoma lines. *Nutrients.* 2018;10(7):1-21. doi:10.3390/nu10070841

159. Tyszka-Czochara M, Bukowska-Strakova K, Majka M. Metformin and caffeic acid regulate metabolic reprogramming in human cervical carcinoma SiHa/HTB-35 cells and augment anticancer activity of Gispilatin via cell cycle regulation. *Food Chem Toxicol.* 2017;106(Pt A):260-272. doi:10.1016/j.fct.2017.05.065

160. Tyszka-Czochara M, Konieczny P, Majka M. Caffeic acid expands anti-tumor effect of metformin in human metastatic cervical carcinoma HTB-34 cells: implications of AMPK activation and impairment of fatty acids de novo biosynthesis. *Int J Mol Sci.* 2017;18(2):1-16. doi:10.3390/ijms18020462

161. Tyszka-Czochara M, Lasota M, Majka M. Caffeic acid and metformin inhibit invasive phenotype induced by TGF-β1 in C-41 and HTB-35 SiHa human cervical squamous carcinoma cells by acting on different molecular targets. *Int J Mol Sci.* 2018;19(1):1-19. doi:10.3390/ijms19010266

162. Al-Hazzani AA, Alsharawi AA. Catechin hydrate inhibits proliferation and mediates apoptosis of SiHa human cervical cancer cells. *Food Chem Toxicol.* 2011;49(12):3281-3286. doi:10.1016/j.fct.2011.09.023

163. Horie N, Hirabayashi N, Takahashi Y, Miyauuchi Y, Taguchi H, Takeishi K. Synergistic effect of green tea catechins on cell growth and apoptosis induction in gastric carcinoma cells. *Biol Pharm Bull.* 2005;28(4):574-579. doi:10.1248/bp.28.574

164. Trybus W, Król T, Trybus E, Stachowska A, Król G. The potential anti-tumor effect of chrysophanol in relation to cervical cancer cells. *J Cell Biochem.* 2021;1-14. doi:10.1002/jcb.29891

165. Taiwo BJ, Popoola TD, van Heerden FR, Fatokun AA, Panagatolylglucose FAA. Pentagalloylglucose, isolated from the leaf extract of Anacardium occidentale L., could elicit rapid and selective cytotoxicity in cancer cells. *BMC Complement Med Ther.* 2020;20(1):287. doi:10.1186/s12906-020-03075-3

166. Sun GP, Wang H, Shen YX, SY X. Inhibitory effects of paeonol on the proliferation of four tumor cell lines. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi.* 2019;35(2):140-145.

167. Zhang L, Zhang S. Modulating Bcl-2 family proteins and Crude Extract of *Rhamnus sphaerocarpa* var. *pabescent* Induce Mixed Cell Death, Increase in Oxidative Stress, DNA Damage, and Inhibition of AKT in Cervical and Oral Squamous Carcinoma Cell Lines. *Oxid Med Cell Longev.* 2018;2018:1-18. doi:10.1155/2018/2390234

168. Moreira TF, Sorbo JM, Souza FdeO, et al. Emodin, Physcion, and Crude Extract of *Rhamnus sphaerocarpa* var. *pabescent* Induce Cell Death, Increase in Oxidative Stress, DNA damage, and Impaired Activation of AKT in Cervical and Oral Squamous Carcinoma Cell Lines. *Biochem Biophys Res Commun.* 2004;8:85-86.

169. Srivinas G, Anto RJ, Srivinas P, Vidhyalakshmi S, Senan VP, Karunagaran D. Emodin induces apoptosis of human cervical cancer cells through poly(ADP-ribose) polymerase cleavage and activation of caspase-9. *Eur J Pharmcol.* 2003;473(2-3):117-125. doi:10.1016/S0014-2999(03)01976-9

170. Thacker PC, Karunagaran D. Curcumin and emodin down regulates TGF-β signaling pathway in human cervical cancer cells. *PLoS One.* 2015;10(3):e012045. doi:10.1371/journal.pone.012045

171. Yaoxian W, Hui Y, Yunyan Z, Yanqin L, Xin G, Xiaoke W. Emodin induces apoptosis of human cervical cancer HeLa cells via intrinsic mitochondrial and extrinsic death receptor pathway. *Cancer Cell Int.* 2013;13(1):71. doi:10.1186/1475-2867-13-71

172. Aborehab NM, Osama N. Effect of gallic acid in potentiating chemotherapeutic effect of paclitaxel in HeLa cervical cancer cells. *Cancer Cell Int.* 2019;19:154. doi:10.1186/s12935-019-0868-0

173. Shi I, Lei Y, Srivastava R, Qin W, Chen JJ. Gallic acid induces apoptosis in human cervical epithelial cells containing human papillomavirus type 16 episomes. *J Med Virol.* 2016;88(1):127-134. doi:10.1002/jmv.24291

174. Zhao B, Hu M. Gallic acid reduces cell viability, proliferation, invasion and angiogenesis in human cervical cancer cells. *Onco Lett.* 2013;6(6):1749-1755. doi:10.3892/ol.2013.1632

175. Fang I, Liu M, Cai L. Hederagenin inhibits proliferation and induces apoptosis of cervical cancer C-4I and HTB-35/SiHa human cervical squamous carcinoma cells. *Int J Mol Med.* 2018;40(6):1669-1678. doi:10.3892/ijmm.2017.3163

176. Kashafi E, Moradzadeh M, Mohamadkhani A, Erfanian S. Kaempferol increases apoptosis in human cervical cancer HeLa cells via PI3K/Akt and telomerase pathways. *Biomed Pharmacother.* 2017;89:573-577. doi:10.1016/j.biopha.2017.02.061

177. Kim H-J, Cho H-S, Ban HS, Nakamura H. Suppression of HIF-1α accumulation by betulinic acid through proteasome degradation in hypoxic cervical cancer. *Biochem Biophys Res Commun.* 2020;523(3):726-732. doi:10.1016/j.bbrc.2020.01.031

178. Xu T, Pang Q, Wáng Y, Yán X. Betulinic acid induces apoptosis by regulating PI3K/Akt signaling and mitochondrial pathways in human cervical cancer cells. *Int J Mol Med.* 2017;40(6):1669-1678. doi:10.3892/ijmm.2017.3163

179. Xu T, Pang Q, Zhou D, et al. Proteomic investigation into betulinic acid-induced apoptosis of human cervical cancer HeLa cells via intrinsic mitochondrial and extrinsic death receptor pathways. *PLoS One.* 2014;9(8):e105768. doi:10.1371/journal.pone.0105768

180. Ip S-W, Weng Y-S, Lin S-Y, et al. The role of Ca+2 on rhein-induced apoptosis in human cervical cancer Ca Ski cells. *Anticancer Res.* 2007;27(1A):379-389.

181. 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

182. Clemente-Soto AF, Salas-Vidal E, Milán-Pacheco C, Sánchez-Carranza JN, Peralta-Zaragoza O, González-Mayá 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

183. 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

184. 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

185. Li X-M, Liu J, Pan F-F, Shi D-D, Wen Z-G, Yang P-L. Quercetin and aconitine synergistically induces the human cervical carcinoma HeLa cell apoptosis via endoplasmic reticulum (ER) stress pathway. PLoS One. 2018;13(1):e0191062. doi:10.1371/journal.pone.0191062

186. 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

187. Alvarez-Sala A, Attanzio A, Tesoriere L, Garcia-Llatas G, Barberá R, Cilla A. Apoptotic effect of a phytosterol-ingredient and its main phytosterol (β-sitosterol) in human cancer cell lines. Int J Food Sci Nutr. 2019;70(3):323-334. doi:10.1080/09637486.2018.1511689

188. Cheng D, Guo Z, Zhang S. Effect of β-sitosterol on the expression of HPV E6 and p53 in cervical carcinoma cells. Contemp Oncol. 2015;19(1):36-42. doi:10.5114/wco.2015.50011