

## ARTICLE

# Bioinformatics Analysis on Molecular Mechanism of Green Tea Compound Epigallocatechin-3-Gallate Against Ovarian Cancer

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Epigallocatechin-3-gallate (EGCG) is the most abundant and biologically active catechin in green tea, and it exerts multiple effects in humans through mechanisms that remain to be clarified. The present study used bioinformatics to identify possible mechanisms by which EGCG reduces the risk of ovarian cancer. Possible human protein targets of EGCG were identified in the PubChem database, possible human gene targets were identified in the National Center for Biotechnology Information database, and then both sets of targets were analyzed using Ingenuity Pathway Analysis (IPA). The results suggest that signaling proteins affected by EGCG in ovarian cancer, which include JUN, FADD, NFKB1, Bcl-2, HIF1 $\alpha$ , and MMP, are involved primarily in cell cycle, cellular assembly and organization, DNA replication, etc. These results identify several specific proteins and pathways that may be affected by EGCG in ovarian cancer, and they illustrate the power of integrative informatics and chemical fragment analysis for focusing mechanistic studies.

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## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ EGCG, a bioactive compound in green tea, is the most abundant and biologically active catechin, and it exerts multiple effects in humans through mechanisms that remain to be clarified.

### WHAT QUESTION DID THIS STUDY ADDRESS?

✓ The present study used bioinformatics to identify possible mechanisms by which EGCG reduces the risk of ovarian cancer.

### WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✓ The results suggest that signaling proteins affected by EGCG in ovarian cancer, which include JUN, FADD,

NFKB1, Bcl-2, HIF1 $\alpha$ , and MMP, are involved primarily in cell cycle, cellular assembly and organization, DNA replication, etc. These results identify several specific proteins and pathways that may be affected by EGCG in ovarian cancer, and they illustrate the power of integrative informatics and chemical fragment analysis for focusing mechanistic studies.

### HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

✓ Verification of targets of EGCG affecting ovarian cancer may provide theoretical guidance for the new drug discovery to ovarian cancer.

Ovarian cancer is a leading cause of death from gynecological cancer in women. Statistical analysis reveals that ovarian cancer is the fifth leading cause of cancer-related mortality in women worldwide.<sup>1</sup> Lacking clearly definable symptoms, ovarian cancer often remains undetected until found at an advanced stage.

In an early stage, only 20% of the new cases of ovarian cancer can be detected and 5-year survival rates are roughly 30% of all of the women diagnosed with advanced-stage ovarian cancer.<sup>2</sup>

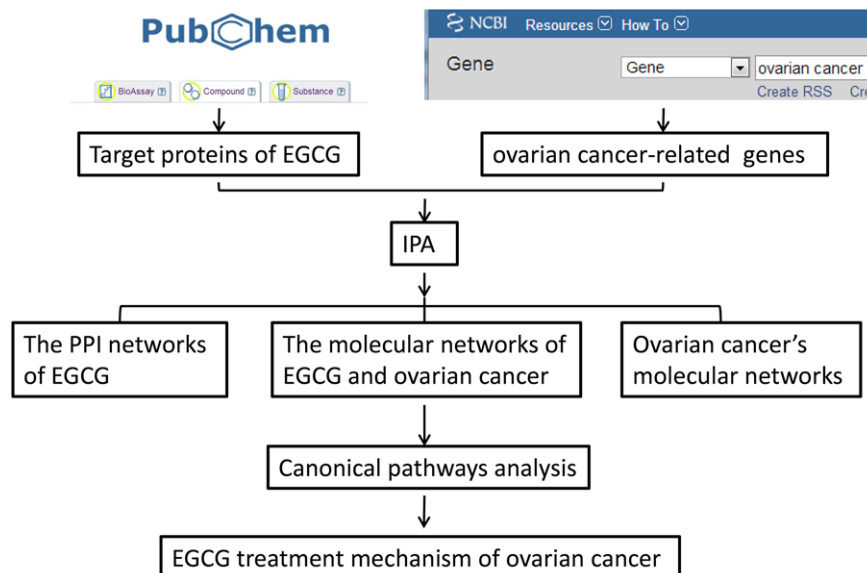
Since the mid-1980s, the association between tea consumption and ovarian cancer has been investigated in many countries.<sup>3–8</sup> Numerous cohort studies and case-control

studies in China,<sup>7</sup> Australia,<sup>4</sup> and the United States<sup>9</sup> have confirmed an association between tea consumption and reduction of ovarian cancer risk. For example, in a 3-year prospective cohort study in China<sup>7</sup> involving 254 patients, the researcher found that increasing the consumption of green tea has a direct proportion with enhanced ovarian cancer survival and reducing the risk of developing ovarian cancer.

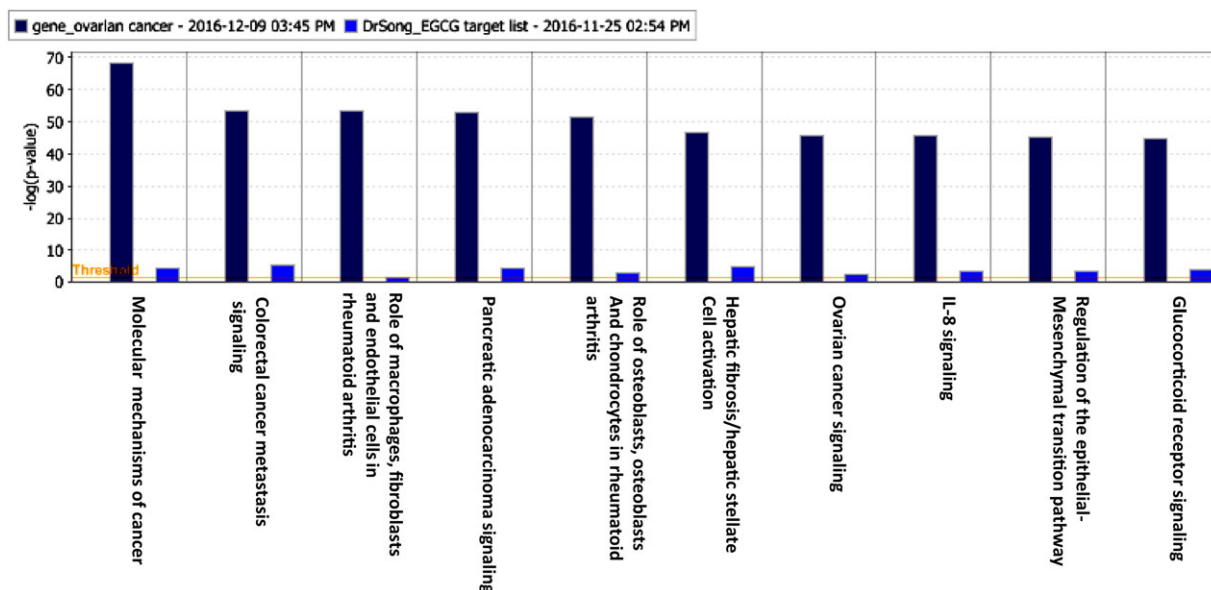
The ability of green tea to protect against ovarian cancer seems to be mediated by catechins, which are polyphenols accounting for 30–40% of the dry weight of brewed green tea. The four major catechins in green tea are (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin,

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**Figure 1** Flow diagram of network-based analysis of possible molecular mechanisms of epigallocatechin-3-gallate (EGCG) in reducing risk of ovarian cancer. IPA, Ingenuity Pathway Analysis; PPI, protein-protein interactions.



**Figure 2** Overlap between signaling pathways linked to ovarian cancer (dark blue) and signaling pathways targeted by epigallocatechin-3-gallate (light blue). Details are listed in **Supplementary Figure S3**. Data were analyzed through the use of QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity).

(-)-epicatechin gallate, and (-)-epicatechin, and EGCG is both the most abundant and most biologically active.<sup>10–13</sup>

Several studies have shown that green tea consumption is associated with a reduced risk<sup>5,14–16</sup> and can enhance the survival of patients with epithelial ovarian cancer.<sup>17</sup> More and more growing evidence suggests that green tea might provide benefit in the treatment of ovarian cancer.

The anticancer properties of green tea are a result of induction of G1 arrest and apoptosis as well as regulation of cell cycle-related proteins in ovarian cancer cell lines.<sup>18</sup> Another study shows that EGCG treatment led to enhanced intracel-

lular hydrogen peroxide and enhances the sensitivity to cisplatin in ovarian cancer cell lines.<sup>19</sup> One or several of these mechanisms may help explain how green tea extracts reduce the risk of ovarian cancer.

The present study explored possible downstream proteins and pathways that EGCG may affect in an effort to guide more detailed mechanistic studies to elucidate how EGCG reduces the risk of ovarian cancer. This study used integrative bioinformatics analysis to bring together predictions of protein and pathway targets, followed by Ingenuity Pathway Analysis (IPA) to build these predicted targets into a network

model of interacting molecules that may help explain the presumably complex effects that green tea exerts in ovarian cancer.

## MATERIALS AND METHODS

### Data set of ovarian cancer-related genes

The National Center for Biotechnology Information Gene Database (<http://www.ncbi.nlm.nih.gov/gene>; up to 15 December 2016), which integrates information from a wide variety of species, was searched for genes related to breast cancer using the search term “ovarian cancer.” Search hits were filtered to retain only Homosapien genes (**Supplementary Table S1**).

### Data set of epigallocatechin-3-gallate-targeted proteins

The PubChem database of small molecules (<http://www.pubchem.ncbi.nlm.nih.gov>; up to 15 December 2016), including the Compound, Bioassay, and Substance subdatabases, was searched for proteins shown in bioassays to be affected by EGCG (CID: 65064) or predicted to be affected by EGCG based on similarity with known binders. Search hits were limited to Homosapien proteins (**Supplementary Table S2**).

### Prediction of interaction networks affected by epigallocatechin-3-gallate

A network of interacting molecules was built using on-line IPA (<http://www.ingenuity.com>) based on the data set of human genes related to ovarian cancer and the data set of EGCG-targeted human proteins (“focus molecules”). Based on the functions of these focus molecules, IPA generated a set of networks likely to be affected by EGCG. Molecules were represented as nodes with different shapes depending on their function, and lines were drawn between nodes shown to be biologically related in at least one reference from the literature, a textbook, or other canonical information stored in the Ingenuity Knowledge Base.

The networks generated by IPA were scored according to the significance of the molecules in the network, then the “Compare” module within IPA was used to determine the significance of the association between focus molecules and canonical pathways, based on Fisher’s exact test. Finally, we overlaid the two networks to discover the most likely targets of EGCG in ovarian cancer (**Figure 1**).

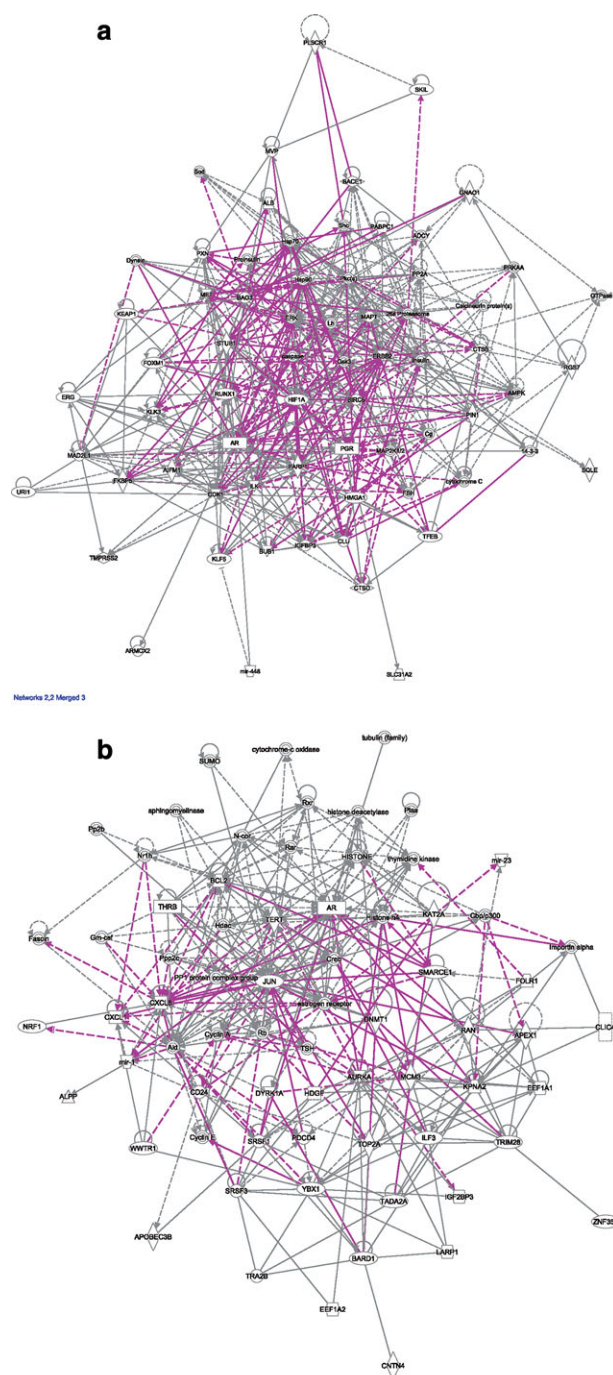
## RESULTS

### Ovarian cancer-related gene networks and their functions

A total of 1,380 human genes linked to ovarian cancer were identified in the GenBank database (**Supplementary Table S1**), and the encoded proteins were assembled into a set of 23 networks using IPA. These pathways involve primarily Cell Death and Survival, Cellular Growth and Proliferation, Cellular Development, Endocrine System Disorders, Cellular Movement, as well as Organism Injury and Abnormalities (**Supplementary Table S3** and **Supplementary Figure S1**).

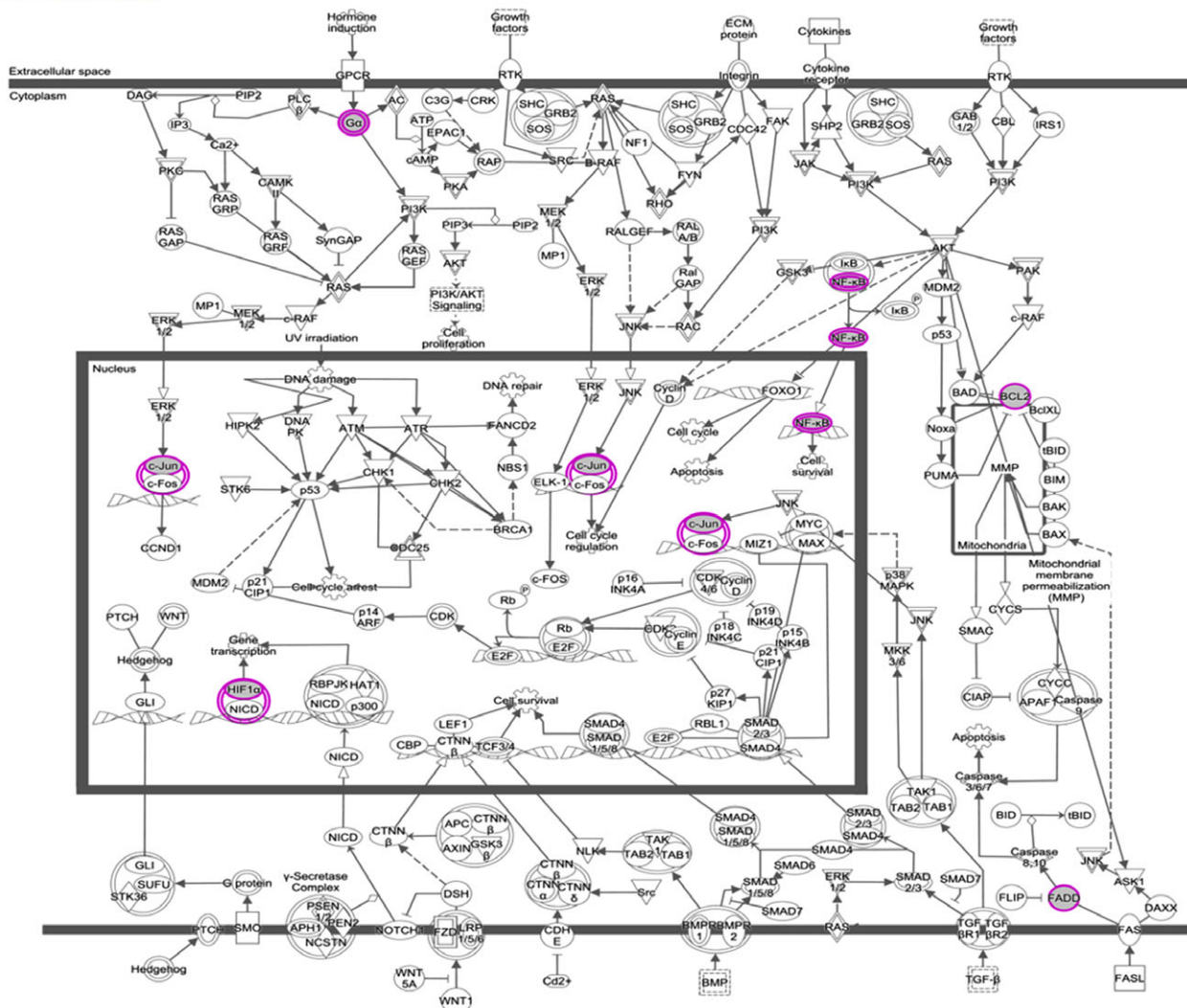
### Epigallocatechin-3-gallate-targeted protein networks and their functions

A total of 65 human proteins targeted by EGCG were identified from the PubChem database (**Supplementary Table S2**)



**Figure 3** Overlap between networks linked to ovarian cancer and networks targeted by epigallocatechin-3-gallate (EGCG). Proteins linked to ovarian cancer are represented as black lines; EGCG-targeted proteins, as purple lines. (a) Shared networks involved in cell death and survival, cellular growth and proliferation, cellular development, cell morphology, nervous system development and function, organ morphology. (b) Shared networks involved in cellular development, cellular growth and proliferation, protein synthesis, cell cycle, cancer, endocrine system disorders. Data were analyzed through the use of QIAGEN’s Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, [www.qiagen.com/ingenuity](http://www.qiagen.com/ingenuity)).





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**Figure 4** Signaling pathways assigned to the Ingenuity Pathway Analysis (IPA) category “molecular mechanisms of cancer” that have been linked to ovarian cancer and targeted by epigallocatechin-3-gallate (EGCG). Proteins directly targeted by EGCG are represented as purple boxes. Data were analyzed through the use of QIAGEN’s Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity).

and their GenInfo Identifier numbers were imported into IPA, which generated protein-protein interaction networks (**Supplementary Table S4** and **Supplementary Figure S2**). Proteins targeted by EGCG participate primarily in the cell cycle, cellular assembly and organization, DNA replication, recombination and repair, cell death and survival, gastrointestinal disease, hepatic system disease, cell morphology, nervous system development and function, organ morphology, and carbohydrate metabolism.

**Network overlap to predict pathways affected by epigallocatechin-3-gallate in ovarian cancer**

The “Canonical Pathway” module of IPA identified 449 signaling pathways linked to ovarian cancer and 246 targeted by EGCG, with 232 signaling pathways shared between the two sets. These overlapping pathways primarily involve molec-

ular mechanisms of cancer, inflammation, and cytokine signaling (**Figure 2**).

The “Networks” module of IPA identified 23 networks linked to ovarian cancer and 6 targeted by EGCG, with 6 networks shared between the two sets (**Figure 3** and **Supplementary Table S5**). These networks are primarily involved in Cell Death and Survival, Cellular Growth and Proliferation, Cellular Development, Cell Morphology, Organ Morphology, DNA replication, recombination and repair, cell cycle, cellular assembly and organization, post-transcriptional modifications, and protein synthesis.

**Prediction of specific epigallocatechin-3-gallate target proteins in ovarian cancer**

Proteins linked to ovarian cancer and targeted by EGCG participate in several canonical pathways underlying a range of

biological activities. To demonstrate the ability of this integrative bioinformatics approach to propose specific protein targets for detailed mechanistic studies, we selected one pathway in the IPA category “molecular mechanisms of cancer” that was linked to ovarian cancer and targeted by EGCG. Several nodes in this pathway emerged as potential direct targets of EGCG in ovarian cancer: JUN, FADD, NFKB1, Bcl-2, HIF1 $\alpha$ , and MMP (Figure 4 and Supplementary Table S6). These potential target molecules also appeared in other canonical pathways linked to ovarian cancer and targeted by EGCG (Supplementary Figure S4 and Supplementary Figure S5).

## DISCUSSION

In this study, we applied an integrative bioinformatics approach drawing on publicly available databases of genes linked to ovarian cancer and of proteins or proteins known or predicted to be affected by EGCG. Based on the large size of both databases, numerous signaling pathways and networks were identified to be potentially linked to ovarian cancer and potentially regulated by EGCG. Then we were able to predict several specific proteins likely to be affected by EGCG in ovarian cancer. These are strong leads for detailed mechanistic studies, illustrating the power of this bioinformatics-based “screening” to guide studies of how EGCG may help reduce the risk of ovarian cancer.

Our network analysis implicated several pathways by which EGCG may reduce ovarian cancer risk, involving cell death and survival, cellular growth and proliferation, cellular development; cell morphology, organ morphology, DNA replication, recombination and repair, cell cycle, cellular assembly and organization, post-transcriptional modifications, and protein synthesis.

These results are consistent with several studies *in vitro* and *in vivo* suggesting that EGCG exerts anticarcinogenic activity by inhibiting cell proliferation via DNA synthesis reduction and inducing apoptotic cell death via DNA damage.<sup>20–24</sup>

Our results are also consistent with work suggesting that EGCG suppresses cell signalization, cell motility, and angiogenesis by inhibiting the phosphorylation of c-Jun and NF- $\kappa$ B, which leads to the overall vascular endothelial growth factor downregulation<sup>25–28</sup> and decreasing the secretion of MMP2 and MMP9.<sup>29–31</sup>

Our analysis of canonical pathways in IPA suggests that EGCG may reduce ovarian cancer risk by altering pathways involved in molecular mechanisms of cancer, inflammatory signaling, and cytokine signaling. In particular, we identified JUN, FADD, NFKB1, Bcl-2, HIF1 $\alpha$ , and MMP as potential targets of EGCG in ovarian cancer.

In agreement with our results, EGCG has been shown to inhibit the phosphorylation of c-Jun and NF- $\kappa$ B, as well as decreasing the secretion of MMP2 and MMP9. The remaining targets that we identified have not previously been reported in the literature, to the best of our knowledge. This means that they may be novel potential targets, such as FADD, Bcl-2, and HIF1 $\alpha$ , that merit validation and, if positive, detailed mechanistic studies.

## CONCLUSION

Integrative bioinformatics analysis and chemical fragment analysis is a promising method for *in silico* “panning” or “screening” of proteins and pathways that EGCG may affect and thereby reduced risk of ovarian cancer. This approach may be suitable for analyzing the mechanism of action of other bioactive compounds. Our network analysis allowed us to identify several pathways by which EGCG may reduce ovarian cancer risk; these pathways are involved mainly in cell death and survival, cellular growth and proliferation, cellular development, cell morphology, organ morphology, DNA replication, recombination and repair, cell cycle, cellular assembly and organization, post-transcriptional modifications, and protein synthesis. Our network analysis also allowed us to identify several specific proteins that EGCG may help regulate in ovarian cancer, including JUN, FADD, NFKB1, Bcl-2, HIF1 $\alpha$ , and MMP.

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**Author Contributions.** S.X. wrote the manuscript. S.X. and L.Y.M. designed the research. Z.M., C.L., and L.Y.M. analyzed the data.

**Conflict of Interest.** The authors declared no conflict of interest.

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