Mechanism of E Lian Granule Reversing Chronic Atrophic Gastritis With Intestinal Metaplasia Based on Integrated Pharmacology and GEO Gene Chip

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Research

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Abstract

**Background:** This study aimed to explore the main components and targets of E-Lian granule through which it reversed chronic atrophic gastritis with intestinal metaplasia, based on the traditional Chinese Medicine Integrated Pharmacology Network Computing Research Platform V2.0 (TCMIP V2.0) combined with GEO gene chips. It also aimed to construct various networks to predict and analyze the mechanism of E-Lian granule in treating gastric precancerous lesions.

**Methods:** The effective traditional Chinese medicine components and targets of E-Lian granule prescription were obtained using TCMIP V2.0. The disease targets were collected using the TCMIP V2.0 platform and the verified gene chips in the GEO database, and the “drug components–targets” network, “compound–targets protein interaction network,” and “core compound targets–pathways network” were constructed using Cytoscape 3.6.1. The reliability of the predicted components and targets was verified using Pymol 1.7.2.1 and Autodock Vina 1.1.2 reverse molecular docking.

**Results:** A total of 262 unique active components and 680 potential active targets of E-Lian granule were obtained. Moreover, 2247 unique disease targets of chronic atrophic gastritis with intestinal metaplasia were obtained by searching the “Disease/Symptom Target Database” combined with the GEO chip (GSE78523) and GeneCard database. Further, 178 complex targets and 38 complex core targets were obtained using Venn and Filter, respectively, such as ALB, TNF, PTGS2, RHOA, ESR1, HRAS, JUN, FOS, CASP3 and so forth. The GO and KEGG enrichment analyses showed that E-Lian granule reversed gastric precancerous lesions not only through the direct intervention of the cancer pathway, gastric cancer pathway, and epithelial signal transduction in *Helicobacter pylori* infection but also through PI3K/AKT, VEGF, MAPK, cAMP, cGMP, Th1/Th2, and other pathways. It also had a significant correlation with cholinergic, 5-hydroxytryptamine, dopaminergic, and other gastrointestinal hormone-related signals. Finally, the core target verified in the GSE78523 chip was successfully used to dock with the active components of E-Lian granules. The reliability of the prediction was also verified.

**Conclusions:** The components and molecular mechanism of E-Lian granule in reversing chronic atrophic gastritis with intestinal metaplasia were predicted by integrated pharmacology, GEO chip, and reverse molecular docking, providing an important theoretical basis for further study of the effective substances and mechanism of E-Lian granule in treating chronic atrophic gastritis.

1. **Background**

Chronic atrophic gastritis (CAG) is a common clinical digestive disease, which is mostly related to *Helicobacter pylori* infection, age, diet, immunity, excessive use of proton pump inhibitors, and other factors. CAG gradually leads to complete or incomplete intestinal metaplasia (IM), which is a precancerous lesion of gastric cancer, because of glandular atrophy. The incidence rate of CAG is as high as 3.77 cases/1000 person/year. Therefore, the reversal of CAG + IM is of great significance for the prevention and treatment of gastric cancer.
E-Lian granule (E-Lian Ke Li, ELKL) is a hospital preparation created by Professor Cai Gan of the first-session famous traditional Chinese medicine (TCM) in Shanghai. It is composed of *Codonopsis pilosula*, *Poria cocos*, *Atractylodes macrocephala*, licorice, *Pinellia ternata*, tangerine peel, *Coptis chinensis*, dandelion, *Hedyotis diffusa*, zedoary, *Angelica sinensis*, and *Salvia miltiorrhiza*. It is a special formulation for treating CAG with IM.

Professor Cai Gan, a mentor, concluded that the core pathogenesis of gastric precancerous lesions was the weakness of spleen and stomach, a combination of blood stasis and heat, based on nearly 60 years of clinical experience. Therefore, the prescription was formulated by replenishing qi, invigorating spleen and resolving phlegm, clearing heat, detoxifying, and activating blood circulation. In a randomized, controlled clinical study, the improvement in TCM syndrome score and pathological inflammation, atrophy, and IM of gastric mucosa biopsy was superior to that in the control group (*P* < 0.05 or *P* < 0.01) and no adverse reactions were noted. Previous experimental studies found that this prescription (the former name of E-Lian granule was Le wei decoction) could reverse precancerous lesions by improving the ability of antioxidation, inhibiting cell proliferation, and affecting the expression of oncogenes.

However, at present, the specific mechanism of E-Lian granule against gastric precancerous lesions has not been totally clarified and needs to be further explored. Both human diseases and TCM compound prescription belong to a complex system. TCM compound prescription acts on the human body through “multicomponents and multitargets”; however, the material basis and mechanism of its effective components are not clear enough because of its complexity, bringing difficulties to the modern research of TCM. In recent years, with the rise in the new discipline of network pharmacology of TCM put forward by Professor Li Shao, the compound prescription of TCM can mine and predict the composition, target, and mechanism of TCM through a variety of methods, such as network high-throughput combinatorial data, computer virtual computing, and network topology analysis strategy. However, it may have a direct impact on the research results due to the uneven data quality and screening criteria of all kinds of TCM and diseases and target database platforms. Therefore, it is necessary to integrate and unify the existing data as much as possible to achieve better research.

The traditional Chinese Medicine Integrated Pharmacology Network Computing Research Platform (TCMIP, http://www.tcmip.cn/) of the Chinese Academy of Traditional Chinese Medicine came into being based on this idea. It is an intelligent data mining platform that integrates big data's management and integrated pharmacological computing services. The experience of famous doctors can be better inherited and developed, and the micromechanism behind the prescription by famous doctors of TCM can be explored, through all kinds of module integration and system integration. At present, many high-quality research papers have been published using this platform. With the help of TCMIP v2.0 platform, GEO database, and molecular docking technology, the disease targets were collected; and “herbs–active components Network”, “Complex Targets Protein Interaction Network” and other multidimensional network correlation analysis were constructed to provide a reference for further exploring the exact mechanism of reversing CAG with IM and clinical new drug research and development. This study discussed the following: first, the TCM experience prescription of the national...
authoritative TCM scholars; second, solid clinical research conclusions in the early stage; third, the first study using TCMIP combined with the GEO database, using experimentally verified gene chip and TCMIP research platform to make the prediction results more reliable; and fourth, the first study of integrated pharmacology of CAG + IM.

2. Materials And Methods

2.1 Construction of Database of Potential Active Components of TCM in the E-Lian Granules

The TCMIP V2.0 platform contains information on the composition of more than 13,000 chemicals in the TCM database, including the ingredient name, Chemical Abstracts Service (CAS) number, Chinese name, English name, molecular formula, relative molecular weight, physical and chemical properties, drug metabolic properties, pharmacological action, and so forth. The data on the active ingredients of 12 TCMs, such as *C. pilosula*, *P. cocos*, *A. macrocephala*, licorice, *Pi. ternata*, tangerine peel, *Cop. chinensis*, dandelion, *H. diffusa*, Rhizoma Curcumae, *A. sinensis*, and *S. miltiorrhiza*, were searched to establish a database of chemical components of the E-Lian granules.

2.2 Construction of E-Lian Granule Herbs–Targets and Disease–Targets Database

The structural similarity between the two-dimensional structure of each active ingredient of TCM and the certified drug in the DrugBank was searched using MedChem (version Studio3.0) software on the platform of TCMIP V2.0. It was concluded that the Tanimoto coefficient >0.8 was the potential active targets of the herbs. Several large public disease databases, such as DisGeNet, OMIM, HPO, ORPHANET, and so on, were integrated in the TCMIP V2.0 “Disease/Symptom Target Database.” The terms “chronic atrophic gastritis” and “intestinal metaplasia” were searched to obtain disease targets, and at the same time, the relevant gene chips were integrated in the GEO database to establish a CAG + IM disease targets database. The same search names were used to search the database, extract differential genes, and integrate the disease target of CAG + IM to obtain a more reliable disease target.

2.3 Construction of Protein–Protein Interaction Network

The potential effective drug targets of ELKL were matched with the disease targets of CAG + IM, the compound targets of “traditional Chinese medicine-disease” were obtained, and then the Venn diagram was drawn using Bioinformatics (http://bioinformatics.psb.ugent.be/webtools/Venn/). Subsequently, the composite target was introduced into the String online tool (https://string-db.org/) to obtain the target protein–protein interaction (PPI) network. After introducing into Cytoscape 3.6.1, the core compound targets were selected through three parameters of the topological network: degree centrality (DC), closeness centrality (CC), and betweenness centrality (BC).

2.4 Enrichment Analysis of GO and KEGG
After obtaining the core compound targets, the gene symbol was converted into Ensembl ID through the Uniprot database (http://www.uniprot.org/), imported into the website OmishareTools (http://www.omicshare.com/tools/index.php/) for GO enrichment function and KEGG enrichment analysis, and finally screened by the P value. GO enrichment mainly analyzed the biological process, cellular composition, and molecular function of the target, while KEGG enrichment could study the potential biological pathways and functions involved in the target.

### 2.5 Construction of Multiple-Associated Networks

The “drug components–targets” network, “compound–targets protein interaction network,” and “core compound targets–pathways network” of E-Lian granules could be constructed and visualized using Cytoscape 3.6.1 software.

### 2.6 Reverse Molecular Docking

The core compound targets were selected to match the verified disease targets in GEO, and then the targets verified by the GEO chip were selected for “components–targets” docking to verify the accuracy of the active components of TCM in E-Lian granules and the predicted targets. The candidate composition and the target crystal structure with ligand binding less than 3Å from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) and RCSB protein data (http://www.pdb.org/), respectively, were downloaded; then, the crystal was imported into Pymol1.7.2.1 software to remove water and separate ligands; and then, AutoDockTools 1.5.6 was imported to construct the docking activity pocket of each target.

Finally, Autodock Vina 1.1.2 software was used to complete docking, and the molecule with the lowest binding energy was selected in the docking conformation to observe the binding effect by matching with the original ligand. The research flow chart is shown in Figure 1.

### 3. Results

#### 3.1 Prediction of Active Components of E-Lian Granules

By searching the Traditional Chinese Medicine Ingredients Database on the TCMIP v2.0 platform, 317 active ingredients with targets for each drug of E-Lian granules were collected. These included 35 components of *C. pilosula*, 10 components of *A. macrocephala*, 32 components of *P. cocos*, 67 components of licorice, 22 components of *Pi. ternata*, 17 components of tangerine peel, 11 components of *Cop. chinensis*, and 2 components of dandelion were present. Moreover, 37 components of Rhizoma Curcumae, 35 components of *S. miltiorrhiza*, and 36 components of *A. sinensis*. The active components in TCMSP platform (https://tcmspw.com/tcmsp.php) and related published literature were searched because *H. diffusa* was not included in TCMIP. Then, it was introduced into TCMIP v2.0 for target fishing, and the target active components were adopted as the effective active components of *H. diffusa* to maintain the unity of the data. Finally, 13 components were obtained. After removing the repetitive
parts of all the active ingredients mentioned earlier, 262 active ingredients were present in E-Lian granules.

3.2 Prediction of Potential Active Targets of E-Lian Granules

A total of 5972 potential active targets of TCM were collected in the TCM target prediction module of TCMIP v2.0 platform, including 619 targets of *C. pilosula*, 139 targets of *A. macrocephala*, 1493 targets of *P. cocos*, 1071 targets of licorice, 444 targets of *Pi. ternata*, 175 targets of tangerine peel, 78 targets of *Cop. chinensis*, 26 targets of dandelion, 333 targets of *H. diffusa*, 443 targets of Rhizoma Curcumae, 619 targets of *A. sinensis*, and 532 targets of *S. miltiorrhiza*. The repetitive targets of various drugs were deleted, and 680 potential active targets of E-Lian granules were obtained. The “drug ingredient–target” network was constructed using Cytoscape 3.6.1 (Figure 2).

3.3 Targets of CAG with IM

Three CAG disease targets were obtained in TCMIP v2.0 “Disease/Symptom Target Database,” but no disease targets of IM were obtained. Series: GSE78523 gene chip for gastric mucosal biopsy of IM obtained by the GEO database (https://www.ncbi.nlm.nih.gov/geo/)²⁹. IM exists in the process of transdifferentiation of gastrointestinal tissue. Two histological subtypes exist: complete (CIM) and incomplete (IIM), which have a higher gastric cancer progression rate. The mRNA chip contains 45 samples: normal group (*n* = 15) and IM group (*n* = 30), including 9 cases of complete IM unadvanced gastric cancer, 8 cases of complete IM progressive gastric cancer, and 7 cases of incomplete IM unadvanced gastric cancer. Six patients with incomplete progressive gastric cancer were selected for differential gene analysis with Rmur3.6.1 Limma. The condition was that adjust *P* < 0.05 and log2 (fold change) = 0.5. A total of 649 IM targets were obtained, the heat map (the first 20 genes) and volcano map (Figure 3) were drawn, and no repetitive target was found. The disease targets (https://www.genecards.org/) were supplemented in the GeneCard Database due to the lack of disease targets to obtain more accurate results. A total of 639 targets were obtained by “chronic atrophic gastritis” search, and 1482 targets were obtained by “intestinal metaplasia” search. A total of 2247 nonrepetitive CAG + IM disease targets were obtained.

3.4 Construction of “ELKL–CAG+IM” Complex Targets Protein Interaction Network

The potential active targets of E-Lian granule were matched with the targets of atrophic gastritis with IM, and 178 complex targets (http://bioinformatics.psb.ugent.be/webtools/Venn/) were obtained. The “ELKL–CAG+IM” complex targets were selected to draw its PPI network using the String online tool. The condition was the minimum required interaction score: low confidence 0.15 (Figure 4).

3.5 Complex Core–Targets screening

The PPI network of the aforementioned 178 compound targets was imported into Cytoscape 3.6.1, and the CytoNca App module was used to sort and filter according to the median values of DC, BC, and CC
with R3.6.1 nodeFilter1 and nodeFilter2. The first filter condition was: degree = 59, betweenness = 49.73034119, and closeness = 0.6 (The first network consists of 178 nodes and 5663 edges). The second filtering condition was as follows: degree = 57.5, betweenness = 14.84758805, and closeness = 0.786089109 (The second network consists of 80 nodes and 2412 edges). Finally, 38 core compound targets were obtained (The last network consists of 178 nodes and 5663 edges), as shown in Figure 5 and Table 1.

**Table 1 Complex core targets.**
| Gene symbol | Uniprot ID | Protein name                          | Betweenness | Closeness | Degree |
|-------------|------------|---------------------------------------|-------------|-----------|--------|
| ALB         | P02768     | Albumin                               | 1.421904562| 1         | 37     |
| TNF         | P01375     | Tumor necrosis factor                 | 1.421904562| 1         | 37     |
| PTGS2       | P35354     | Prostaglandin G/H synthase 2          | 1.421904562| 1         | 37     |
| RHOA        | P61586     | Transforming protein RhoA             | 1.421904562| 1         | 37     |
| ESR1        | P03372     | Estrogen receptor                     | 1.421904562| 1         | 37     |
| HRAS        | P01112     | GTPase HRas                           | 1.421904562| 1         | 37     |
| JUN         | P05412     | Transcription factor AP-1             | 1.421904562| 1         | 37     |
| FOS         | P01100     | Proto-oncogene c-Fos                  | 1.421904562| 1         | 37     |
| CASP3       | P42574     | Caspase-3                             | 1.421904562| 1         | 37     |
| TLR4        | O00206     | Toll-like receptor 4                  | 1.421904562| 1         | 37     |
| ABCB1       | P08183     | ATP-dependent translocase ABCB1       | 1.421904562| 1         | 37     |
| AKT1        | Q96B36     | Proline-rich AKT1 substrate 1         | 1.421904562| 1         | 37     |
| AR          | P10275     | Androgen receptor                     | 1.421904562| 1         | 37     |
| NR3C1       | P04150     | Glucocorticoid receptor               | 1.421904562| 1         | 37     |
| PGR         | P06401     | Progesterone receptor                 | 1.421904562| 1         | 37     |
| HSP90AA1    | P07900     | Heat shock protein HSP 90-alpha       | 1.421904562| 1         | 37     |
| INS         | P01308     | Insulin                               | 1.421904562| 1         | 37     |
| MAPK3       | P27361     | Mitogen-activated protein kinase 3    | 1.421904562| 1         | 37     |
| ACTB        | P60709     | Actin, cytoplasmic 1                  | 1.421904562| 1         | 37     |
| IL1B        | P01584     | Interleukin-1 beta                    | 1.421904562| 1         | 37     |
| EGF         | P01133     | Pro-epidermal growth factor           | 1.421904562| 1         | 37     |
| IL6         | P05231     | Interleukin-6                         | 1.421904562| 1         | 37     |
| PPARG       | P37231     | Peroxisome proliferator-activated receptor gamma | 1.421904562| 1         | 37     |
| HNF4A       | P41231     | Hepatocyte nuclear factor 4-alpha     | 1.098906787 | 0.973684211 | 36 |
| IL2         | P60568     | Interleukin-2                         | 1.097038971 | 0.973684211 | 36 |
| Gene    | Accession | Description                                                                 | Fold Change | P-value | n   |
|---------|-----------|-----------------------------------------------------------------------------|-------------|---------|-----|
| CYP19A1 | P11511    | Aromatase                                                                   | 1.019309086 | 0.973684211 | 36  |
| DNMT1   | P26358    | DNA (cytosine-5)-methyltransferase 1                                        | 1.098906787 | 0.973684211 | 36  |
| CREB1   | P16220    | Cyclic AMP-responsive element-binding protein 1                             | 0.832091807 | 0.948717949 | 35  |
| NQO1    | P15559    | NAD(P)H dehydrogenase [quinone] 1                                           | 0.897985556 | 0.948717949 | 35  |
| DRD2    | P14416    | D(2) dopamine receptor                                                       | 0.569038583 | 0.925    | 34  |
| SDHC    | Q99643    | Succinate dehydrogenase cytochrome b560 subunit, mitochondrial               | 0.644469618 | 0.925    | 34  |
| SLC2A1  | P11166    | Solute carrier family 2, facilitated glucose transporter member 1           | 0.713067128 | 0.925    | 34  |
| VDR     | P11473    | Vitamin D3 receptor                                                         | 0.770969427 | 0.925    | 34  |
| ALDH1A1 | P00352    | Retinal dehydrogenase 1                                                     | 0.375612386 | 0.902439024 | 33  |
| NFkB1   | P19838    | Nuclear factor NF-kappa-B p105 subunit                                      | 0.373461848 | 0.902439024 | 33  |
| NOS2    | P35228    | Nitric oxide synthase, inducible                                            | 0.706686896 | 0.880952381 | 32  |
| GSTP1   | P09211    | Glutathione S-transferase P                                                 | 0.650935135 | 0.880952381 | 32  |
| CFTR    | Q20BI4    | Cystic fibrosis transmembrane conductance regulator                         | 0.447715054 | 0.860465116 | 31  |

### 3.6 Complex Core Targets GO enrichment analysis

Through GO enrichment analysis, 38 core targets were found to be involved in the cellular composition, molecular function, and biological processes (P value < 0.05), such as positive regulation of nucleobase-containing compound metabolic process (GO:0045935); response to an endogenous stimulus (GO:0009719 in biological processes); RNA polymerase II regulatory region sequence-specific DNA binding (GO:0000977); regulatory region nucleic acid binding (GO:0001067 in molecular processes); and so forth. The first 20 functional enrichment circle diagrams and secondary classification diagrams were selected, as shown in Figure 6.

### 3.7 Complex Core Targets KEGG enrichment analysis

Through KEGG enrichment analysis, the main signaling pathways involved in the treatment of CAG + IM targets with E-Lian granule were identified. Moreover, 20 pathways related to CAG + IM were screened and significantly enriched (P value < 0.05), in addition to the routine inflammatory pathways, such as TNF signaling pathway (hsa04668), MAPK signaling pathway (hsa04010), and PI3K/Akt signaling pathway...
Also, occurrence of CAG+IM was closely related to gastric cancer pathways: pathways in cancer (hsa05200), gastric cancer (hsa05226), epithelial cell signaling in *H. pylori* infection (hsa05120), and gastrointestinal hormone–related signal regulation, such as cholinergic synapse (hsa04725), serotonergic synapse (hsa04726), and dopaminergic synapse (hsa04728). The “core targets–pathways” network diagram was constructed (Figure 7). (The last network consists of 49 nodes and 138 edges.)

### 3.8 Complex Core Targets Reverse Molecular Docking

In 38 core disease prescription–drug compound targets, the core complex targets were mapped with 649 differential genes of GSE78523 chip to obtain three overlapping genes: VDR, HNF4A, and CFTR. HNF4A and CFTR were selected as small-molecule bodies, and they corresponded to six and eight active components of E-Lian granules as ligands, respectively. Myristic acid (*C. pilosula* and *A. sinensis*) and guanosine (*Pi. ternata*) were selected for reverse molecular docking. The crystal structures of 1M7W (HNF4A, containing the original ligand DAO, 2.8) and 2BBT (CFTR, containing the original ligand ATP) were downloaded from the RCSB protein data. HNF4A combines myristic acid, grid center $X = 63.596$, $Y = 29.172$, $Z = 11.49$, NPTs=40 40 40 0.375, and the minimum binding energy of the most stable state of successful docking between the two was affinity $=-5.5$ kcal/mol. CFTR combined with guanosine, grid center $X = 26.419$, $Y = 29.322$, $Z = 99.23$, NPTs=40 40 40 0.375, and the minimum binding energy of the most stable state of successful docking between the two was affinity $=-6.4$ kcal/mol (Figure 8).

### 4. Discussion

CAG with IM is a benign, precancerous lesion with a tendency to develop into gastric cancer, in which IM is divided into complete IM and incomplete IM. Incomplete IM has more overexpressed oncogenes and molecular processes than intact IM, but the difference between histological subtypes of IM with or without GC is less than that of normal mucosa. Therefore, irrespective of the kind of IM, the possibility of canceration exists. A national multicenter cross-sectional study in China showed that the risk factors for CAG were related to not only common *H. pylori* infection, age, smoking, drinking, environmental, and drug factors but also insufficient acid production and pro-inflammatory genetic characteristics in the body. As no clear Western medicine exists for the prevention and treatment of precancerous lesions such as CAG with IM, the role of TCM in preventing precancerous lesions has gradually attracted the attention of researchers all over the world.

Professor Cai Gan, according to Li Dongyuan (ancient Chinese medicine scientists) stated “tonifying the spleen and stomach and dispelling evil fire”, “Evil fire is opposed to human vitality. If the evil fire is hyperactive, the human body will be unhealthy, and if the righteousness is sufficient, the human body will be healthy.” Gastric mucosal atrophy is the manifestation of weakness of spleen and stomach, and IM is caused by damp-heat, blood stasis, and heat for a long time, all belonging to the category of “evil fire.” Professor Cai Gan inherited and developed the theory of ancient Chinese medicine, produced E-Lian granule, took Liujunzi decoction (composed of *C. pilosula*, *A. macrocephala*, *P. cocos*, licorice, and *Pi. ternata*) as the base prescription for invigorating spleen and removing dampness; Radix Salviae
Miltiorrhizae, *A. sinensis*, and Rhizoma Curcumae for promoting blood circulation, clearing heat, and removing blood stasis; and dandelion, *Cop. chinensis*, and *H. diffusa* for clearing heat, detoxification, and dispersing knots. E-Lian granule in the clinical treatment of CAG + IM has been proved so as to reverse gastric precancerous lesions, but the more comprehensive specific mechanism of the compound is not clear.

In this study, network pharmacology, GEO data, and reverse molecular docking were used to explore its microscopic mechanism based on integrated pharmacology platform TCMIP v2.0. TCMIP v2.0 originates from the data resources of the international authoritative database the Encyclopedia of Traditional Chinese Medicine, which integrates the data platform of TCM; integrates disease, syndrome, prescription, and medicine; simplifies complexity; and quickly mines the potential mechanism of famous doctors from different angles. The GEO database is a gene expression database created and maintained by the National Center for Biotechnology Information of the United States. Founded in 2000, it contains high-throughput gene expression data published by research institutions all over the world, all of which have been verified by clinical or experimental data, which increases the reliability of the TCM analysis.

In this study, 262 unique active components and 680 potential active targets of E-Lian granules were obtained using TCMIP V2.0 platform. All the drug ingredients obtained by TCMIP were adopted, but *H. diffusa* had no related data. Therefore, the active components of TCMSP and related literature were used to hang targets on the TCMIP V2.0 platform to obtain more reliable targets. At the same time, 2247 unique disease targets of CAG with IM were obtained by searching “Disease/Symptom Target Database” combined with GEO chip (GSE78523) and GeneCard database. “ELK–CAG+IM” compound targets and 38 core targets were obtained using Venn and Filter, respectively, such as ALB, TNF, PTGS2, RHOA, ESR1, HRAS, JUN, FOS, CASP3, and so forth. Through GO and KEGG enrichment analyses, E-Lian granule could reverse gastric precancerous lesions not only through the direct intervention of cancer pathway, gastric cancer pathway, and epithelial signal transduction in *H. pylori* infection but also through PI3K/AKT, VEGF, MAPK, cAMP, cGMP, Th1/Th2, and other pathways. Some of the conclusions of this study were also included in a similar study. The most important of the empirical prescription Qilian Shupi decoction is the cancer-related pathway (apoptosis, p53, and VEGF) in *H. pylori* infection and epithelial cell signal transduction.36

For example, in the PI3K/Akt pathway, the expression of PI3K and Akt phosphorylation was higher in the gastric precancerous lesion and gastric cancer groups than in the gastritis group, suggesting that the proliferative signal pathway of PI3K/Akt in gastric mucosa could be activated in the process of chronic gastritis–atrophic gastritis–IM–dysplasia–gastric cancer, which might be one of the pathogenic mechanisms of gastric cancer. 37

The infection rate of *H. pylori* was significantly higher in patients with early-stage gastric cancer than in those with advanced-stage gastric cancer (*P* < 0.05). Related experiments proved that *H. pylori* might promote the proliferation of tumor cells by activating the PI3K/Akt pathway in gastric cancer cells.38
With regard to the relationship between VEGF and CAG, related reports confirmed that the VEGF-634G > C polymorphism, especially the GG + GC genotype, was associated with the increased risk of gastric precancerous lesions transforming into gastric cancer and the increased level of VEGF. The prescription of TCM under the principle of invigorating the spleen and regulating qi, activating blood circulation, and removing blood stasis might inhibit abnormal cell proliferation and differentiation of CAG by reducing the expression of ERK1, ERK2, and MAPK1 in the gastric tissue of rats. After analyzing and comparing the expression of VEGF in the IM of gastric mucosa treated with *H. pylori* and eradication therapy, Rui et al. found that *H. pylori* could upregulate the expression of VEGF, but the expression of VEGF could not decrease to normal after the eradication therapy. A study comparing the positive rate of VEGF expression in the gastric mucosa of patients with acute simple gastritis and that of patients with CAG showed that the expression of VEGF in the gastric mucosa of patients with CAG significantly increased, but this was only one of the pathological changes of CAG and was not directly related to the degree of CAG. Some studies also found that moxibustion at stomach meridian acupoints could significantly reduce the expression of proliferation factor VEGF in gastric mucosa of rats with precancerous lesions of CAG, inhibit the atypical proliferation of gastric mucosal cells, and promote the repair of the gastric mucosa.

In terms of apoptosis, the scholars detected the proliferation index (PI), apoptosis index (AI), apoptosis–proliferation ratio, and apoptosis intensity in gastric antrum biopsies of patients with chronic superficial gastritis (CSG), CAG, and CAG + IM, respectively. The results showed a positive correlation between apoptosis and proliferation in CSG and CAG (r = 0.5475 and r = 0.5839), while the PI of CAG + IM PI was the highest. However, a negative correlation was found between apoptosis and proliferation (r = -0.6742). The intensity of apoptosis was extremely low, which was less than that of CSG. The atrophy of CAG might be caused by excessive apoptosis, while CAG + IM showed disturbance of apoptosis and excessive proliferation, which was similar to the cellular biological manifestations of dysplasia and carcinoma. Other related studies showed that precancerous lesions were nonspecific processes characterized by long-term cell degradation and proliferation. However, because it was a benign disease, even if some oncogenes were activated, cell metabolism might still be insufficient, especially nucleic acid metabolism and DNA repair, but these processes were reversible. *B-cell lymphoma-2* (Bcl-2) is one of the most important oncogenes in the study of apoptosis. The low expression of the Bcl-2 gene suggests atrophic gastritis, IM, and intestinal-type gastric adenocarcinoma.

In terms of the molecular mechanism of cAMP and cGMP, team 42 of Zhang Jingren (one of the masters of TCM in China) detected plasma cAMP and cGMP in 87 cases of atrophic gastritis with spleen qi deficiency syndrome. The two indexes of all patients were found to be lower than the normal low limit. Zhang’s Weiwei’an granule (which is also a TCM prescription for treating atrophic gastritis) improved significantly after one or two courses of the treatment. To some extent, it reflected the essence of spleen and stomach qi deficiency of CAG. A report in 2005 showed that acupuncture could increase the content of cAMP in gastric mucosa and reduce the content of cGMP.
Regarding the regulation of Th1/Th2 balance, for example, the Department of Gastroenterology of Hebei Hospital of TCM used the empirical prescription Huazhuo jiedu Hewei recipe (the empirical prescription of Hebei Hospital of TCM for treating atrophic gastritis) to treat CAG for 12 weeks; it upregulated Th2-type cytokine interleukin-4 (IL-4) and decreased the VEGF and EGF levels, but did not affect serum Th1 cytokine interferon-gamma (IFN-γ) level, thus regulating the Th1/Th2 balance. In a study of Shengyang Yiwei decoction (a classical prescription for the treatment of digestive diseases recorded in TCM) and the treatment of CAG rats, opposite results were obtained. Shengyang Yiwei decoction could increase the secretion of IFN-γ and decrease the secretion of IL-4 in gastric tissue and serum of rats, thus regulating the balance of Th1/Th2.

In addition, E-Lian granule might reverse CAG + IM and improve the symptoms of dyspepsia through the brain or gastrointestinal hormone-related signals, such as cholinergic, 5-hydroxytryptamine (5-HT4), and dopaminergic receptors. Dopamine receptor antagonists, such as metoclopramide hydrochloride and domperidone, act directly on the gastrointestinal wall, increase the tension of the lower esophageal sphincter, prevent gastroesophageal reflux, and promote gastric emptying. E-Lian granule could stimulate selective 5-HT4 receptors, such as cisapride and mosapride, and induce the release of acetylcholine by stimulating cholinergic intermediate neurons in the gastrointestinal tract and 5-HT4 receptors in the myenteric plexus, thereby increasing gastrointestinal motility and improving the symptoms of dyspepsia. Previous clinical studies confirmed that the E-Lian granule was effective in improving epigastric pain, epigastric tiredness, and fatigue ($P < 0.05$ or $P < 0.01$).

5. Conclusions

In this study, integrated pharmacology combined with GEO chip was used for the first time to explore the potential effective components and targets of Professor Cai Gan's experience prescription in treating CAG with IM, so as to provide some reference for further experiments to verify the micromechanism of E-Lian granules and contribute to inheriting and carrying forward the experience of mentors.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors agreed to publish the manuscript.

Availability of data and materials

The data used in this study can be obtained from online data platforms, such as TCMIP V2.0 and GEO database, and also from the corresponding authors on reasonable request.
Competing interests

The authors declare no competing interests.

Authors’ Contributions

GSZ and HZH conceived the idea of the manuscript. GSZ and XY wrote the manuscript. XSG, TYN, and WH analyzed the data, and DDB and CG supervised and revised them. All the authors reviewed the final manuscript.

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**Supplementary Materials**

- Supplementary 1. Table S1 ELKL Active Components.
- Supplementary 2. Table S2 Active Targets of ELKL.
- Supplementary 3. Table S3 All the chronic atrophic gastritis with intestinal metaplasia–related targets.
- Supplementary 4. ELKL potential targets Veen CAG+IM–related targets.
- Supplementary 5. Table S4 Core targets screening process
- Supplementary 6. Table S6 Core targets GO enrich
- Supplementary 7. Table S7 Core targets selected 20 CAG+IM related KEGG
- Supplementary 8. Autodocking Binding energy