Original Article

Network and pathway-based analysis of candidate genes associated with esophageal adenocarcinoma

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Background: Previous studies have made some headway in analyzing esophageal adenocarcinoma (EA) with respect to pathogenic factors, treatment methods, and prognosis. However, far less is known about the molecular mechanisms. Thus, a comprehensive analysis focusing on the biological function and interaction of EA genes would provide valuable information for understanding the pathogenesis of EA, which may provide new insights into gene function as well as potential therapy targets.

Methods: We selected 109 genes related to EA by reviewing 458 publications from the PubMed database. In addition, performing gene enrichment assays, pathway enrichment assays, pathway crosstalk analysis, and extraction of EA-specific subnetwork were used to describe the relevant biochemical processes.

Results: Function analysis revealed that biological processes and biochemical pathways associated with apoptotic and metabolic processes, a variety of cancers, and drug reaction pathways. Further, 12 novel genes (*PTHLH*, *SUMO2*, *TYMS*, *APP*, *PTGIR*, *SP1*, *UBC*, *COL1A1*, *GSTO1*, *TRAF6*, *BMP7*, and *RAB40B*) were identified in the EA-specific network, which might provide helpful information for clinical application.

Conclusions: Overall, by integrating pathways and networks to explore the pathogenetic mechanisms underlying EA, our results could significantly improve our understanding of the molecular mechanisms of EA and form a basis for selection of potential molecular targets for further exploration.

Keywords: Esophageal adenocarcinoma; oncogene; function enrichment analysis; pathway crosstalk; protein-protein interaction network

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Introduction

Esophageal cancer, the ninth most common cancer and the sixth most fatal cancer worldwide, is classified into 2 major histologic types, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EA), the latter of which has increased rapidly in Western countries over the past several decades (1). Risk factors for EA include Barrett's esophagus, gastroesophageal reflux disease, Caucasian race, male gender, obesity, smoking, and some genetic factors (2). EA has shown a poor prognosis, with an overall survival rate of approximately 20% at 5 years (3). Recently, with the development of neoadjuvant chemoradiotherapy and radiotherapy, survival rates have improved in patients with locally advanced EA compared to surgery alone, but there is wide interindividual variation in response to

neoadjuvant therapy (4). In recent years, analysis of the genome of patients with EA has become increasingly relevant for guiding treatment planning.

The genomic complexity of EA is characterized by a high burden of point mutations and genome structural alterations, including *TP53*, *CDKN2A*, *KRAS*, *MYC*, and *CDK6* (3). Rapid advances in high throughput technologies over the past decade have helped researchers generate numerous genetic and genomic datasets in order to reveal causal genes and their actions in complex diseases (5). Pathway analysis examines series of actions or interactions among genes or genes products that lead to the generation of a certain product or a change in the cell and protein-protein interaction (PPI) network, and it has been recognized as a powerful tool for understanding how genes perform their biological function (6).

In this study, we conducted a comprehensive analysis of genes which have been published potentially involved in EA. We then performed biological enrichment analyses and the interaction among the enriched biochemical pathways was analyzed, in addition to examining the crosstalk among the significantly enriched pathways. Finally, a molecular network of EA was constructed. We present the following article in accordance with the STREGA reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1286/rc).

Highlight box

Key findings

- We found that biochemical processes and pathways associated with the metabolic process, biological regulation process, and the signal transduction process played key roles in the molecular mechanism of esophageal adenocarcinoma (EA). In addition, a number of new genes were identified in the EA-specific network.

What is known and what is new?

- We know which genes and their pathways have been studied in EA.
- We found that biochemical processes and pathways associated with the metabolic process, biological regulation process, and the signal transduction process played key roles in the molecular mechanism of EA. In addition, there were 2 interconnected pathway modules: metabolic regulation and drug reactions, and transcriptional regulation. Finally, we extracted an EA-specific subnetwork and identified some novel genes potentially bound up with EA.

What is the implication, and what should change now?

- Our results suggested that in future studies in EA, we should focus on the pathways and new genes involved.

Methods

Identification of EA-related genes

The genes genetically associated with EA (the standardized term found though MeSH) were gathered by retrieving human genetic association studies published in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/). We retrieved 458 articles published to June 30, 2022 using the search terms (adenocarcinoma of esophageal and polymorphism [MeSH]) or (adenocarcinoma of esophageal and genotype [MeSH]) or (adenocarcinoma of esophageal and alleles [MeSH]), with 'humans' as the limiting condition. After browsing the abstracts of all of the articles, we excluded those which were not gene-related or were not focused primarily on EA, which left 125 articles remaining. We then focused on studies reporting a significant association between 1 or more genes with EA. For the purpose of reducing the number of potential false-positive genes, the studies reporting negative or insignificant associations were excluded even though some of them might influence EA in further research based on a large number of studies. Finally, we read the full text of each selected article to ensure the conclusion was in accord with its contents, and we also added some genes that were synergistic to the investigated genes. Eventually, we found 109 genes related to EA. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Function enrichment analysis

We used software from the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (https://david.ncifcrf.gov/) to convert the names of 109 EA genes from the literature into Entrez Gene GeneIDs. To examine the functional features of EA genes, Gene Ontology (GO, http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.kegg.jp/) were applied for functional enrichment analysis. In brief, GO enrichment analysis was used to annotate and classify the candidate genes of EA according to molecular function (MF), biological process (BP), and cellular component (CC). In addition, directed acyclic graph (DAG) was used to depict the results of GO enrichment analysis. In DAG, the top 10 terms with the lowest P value in the rectangle and their parent nodes in the circle were shown, with the branches indicating annotation moving from more general to more specific as one moved

from parent nodes to child nodes, and the defined function range became smaller from top to bottom. The terms with pane marks indicated significant enrichment, with more red indicating more significance. We then applied KOBAS 2.1.1 software for comparison with the genes included in each pathway in the KEGG database, which is a knowledge base for systematic analysis of gene functions in order to link genomic information with higher order functional information. Next, we extracted the significantly enriched pathways and assigned a P value for each of them using Fisher's exact test. In our study, both the GO and KEGG biological process terms with a multiple testing correction P value [false discovery rate (FDR)] <0.05 calculated using the Benjamini-Hochberg procedure were considered to be significantly enriched. In addition, we evaluated the degree of gene enrichment in a single function by enrichment score with the formula:

$$R_e = \frac{nf / n}{Nf / N} \quad [1]$$

Pathway crosstalk analysis

We further performed pathway crosstalk analysis to investigate interactions of the enriched pathways. In this study, we used 2 measures to describe the overlap between any 2 pathways, the overlap coefficient (OC) and the Jaccard coefficient (JC).

$$OC = \frac{|A \cap B|}{\min(|A|,|B|)} \text{ and } JC = \frac{|A \cap B|}{|A \cup B|} \quad [2]$$

where A and B are the lists of genes included in the 2 pathways under examination. The following procedure was carried out to construct the pathway crosstalk:

(I) We first selected a set of pathways for crosstalk analysis. Only the pathways with FDR <0.05 were kept. At the same time, more than 5 candidate genes were required in each pathway as pathways with too few genes might not have had sufficient biological information.

(II) Next, we counted the shared candidate genes of each pathway pair and removed pathways with fewer than 3 overlapping genes.

(III) We then calculated the JC and OC of the qualified pathway pairs and ranked them according to their score values.

$$Score = \frac{(JC + OC)}{2} \quad [3]$$

Lastly, we visualized the final pathway crosstalk with Cytoscape software (7). The node size represented the degree of the pathways, with a larger node corresponding to a deeper degree, and the thickness of the line represented the score value of pathways, with a thicker line corresponding to a higher score.

Construction of the EA-specific protein subnetwork

Human PPI data was downloaded from the Protein Interaction Network Analysis (PINA) platform (8), which pools and curates unique physical interaction information from 6 main public PPI databases (IntAct, BioGRID, MINT, DIP, HPRD , and MIPS/MPact). We then used the Klein-Ravi algorithm in GenRev software to extract the subnetwork using the 109 EA genes as seeds (9). To examine the nonrandomness of the constructed network, 1000 random networks with the same number of nodes and edges as the EA-specific network were generated using the Erdos-Renyi model in the igraph package in R. Afterwards, we compared the seed genes with the 1000 random networks to generate subnetworks and calculated the average values of the shortest-path distance and clustering coefficient in random subnetworks. We estimated the significance of nonrandomness by counting the number of random networks with average shortest-path distance (*nL*) less than that of the EA-specific network and the number of random networks with average clustering coefficient (*nc*) more than that of the EA-specific network. Finally, we calculated the *P*-value = *nL*/1000 and *nc*/1000.

Results

Identification of genes related to EA

By searching PubMed, we collected literature on genetic associations related to EA. We selected 125 publications which reported gene(s) significantly associated with EA and collected 109 genes related to EA (*Table 1*). Some of the genes were involved in transcriptional regulation, such as hypoxia-inducible factor-1 (*HIF-1*) signaling (*FLT1*, *BCL2*, *IGF1R*, *VEGFA*, and *PIK3CA*), tumor necrosis factor (*TNF*) signaling (*IL1B*, *MMP14*, and *PTGS2*), vascular endothelial growth factor (*VEGF*) signaling (*CASP9*, *KRAS*), and others

Table 1 List of the 109 EA-related genes

Gene abbreviations	Gene ID	Species	Gene name
CTHRC1	115908	Homo sapiens	Collagen triple helix repeat containing 1 (*CTHRC1*)
FHIT	2272	Homo sapiens	Fragile histidine triad (*FHIT*)
PTGS2	5743	Homo sapiens	Prostaglandin-endoperoxide synthase 2 (*PTGS2*)
GDF7	151449	Homo sapiens	Growth differentiation factor 7 (GDF7)
ASCC1	51008	Homo sapiens	Activating signal cointegrator 1 complex subunit 1 (*ASCC1*)
SELENBP1	8991	Homo sapiens	Selenium binding protein 1 (*SELENBP1*)
MMP3	4314	Homo sapiens	Matrix metallopeptidase 3 (*MMP3*)
XRCC1	7515	Homo sapiens	X-ray repair cross complementing 1 (*XRCC1*)
MMP2	4313	Homo sapiens	Matrix metallopeptidase 2 (*MMP2*)
IL10	3586	Homo sapiens	Interleukin 10 (*IL10*)
MMP1	4312	Homo sapiens	Matrix metallopeptidase 1 (*MMP1*)
PGR	5241	Homo sapiens	Progesterone receptor (*PGR*)
GSTM1	2944	Homo sapiens	Glutathione S-transferase mu 1 (*GSTM1*)
GSTM3	2947	Homo sapiens	Glutathione S-transferase mu 3 (*GSTM3*)
MUTYH	4595	Homo sapiens	mutY DNA glycosylase (*MUTYH*)
CDKN2A	1029	Homo sapiens	cyclin dependent kinase inhibitor 2A (*CDKN2A*)
GATA6	2627	Homo sapiens	GATA binding protein 6 (*GATA6*)
FOXF1	2294	Homo sapiens	Forkhead box F1 (*FOXF1*)
GATA4	2626	Homo sapiens	GATA binding protein 4 (*GATA4*)
IL1B	3553	Homo sapiens	Interleukin 1 beta (*IL1B*)
PIK3CA	5290	Homo sapiens	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*)
NQO1	1728	Homo sapiens	NAD (P)H quinone dehydrogenase 1 (*NQO1*)
WWOX	51741	Homo sapiens	WW domain containing oxidoreductase (*WWOX*)
EGFR	1956	Homo sapiens	Epidermal growth factor receptor (*EGFR*)
NEIL2	252969	Homo sapiens	nei like DNA glycosylase 2 (*NEIL2*)
GSTT1	2952	Homo sapiens	Glutathione S-transferase theta 1 (*GSTT1*)
ARID1A	8289	Homo sapiens	AT-rich interaction domain 1A (*ARID1A*)
CYP2E1	1571	Homo sapiens	Cytochrome P450 family 2 subfamily E member 1 (*CYP2E1*)
CDO1	1036	Homo sapiens	Cysteine dioxygenase type 1 (*CDO1*)
RFC3	5983	Homo sapiens	Replication factor C subunit 3 (*RFC3*)
VEGFA	7422	Homo sapiens	Vascular endothelial growth factor A (*VEGFA*)
HPP1	780897	Homo sapiens	Hyperpigmentation, progressive, 1 (*HPP1*)
FGFR2	2263	Homo sapiens	Fibroblast growth factor receptor 2 (*FGFR2*)
WNT5A	7474	Homo sapiens	Wnt family member 5A (*WNT5A*)
MCL1	4170	Homo sapiens	BCL2 family apoptosis regulator (*MCL1*)
CRTC1	23373	Homo sapiens	CREB regulated transcription coactivator 1 (*CRTC1*)
ERBB2	2064	Homo sapiens	erb-b2 receptor tyrosine kinase 2 (*ERBB2*)
TIMP3	7078	Homo sapiens	TIMP metallopeptidase inhibitor 3 (*TIMP3*)

Table 1 (*continued*)

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Gene abbreviations	Gene ID	Species	Gene name
EPHB4	2050	Homo sapiens	EPH receptor B4 (*EPHB4*)
WT1	7490	Homo sapiens	Wilms tumor 1 (*WT1*)
VDR	7421	Homo sapiens	vitamin D (1,25- dihydroxyvitamin D3) receptor (*VDR*)
KRAS	3845	Homo sapiens	KRAS proto-oncogene, GTPase (*KRAS*)
DMD	1756	Homo sapiens	Dystrophin (*DMD*)
EGF	1950	Homo sapiens	Epidermal growth factor (*EGF*)
RUNX1	861	Homo sapiens	Runt related transcription factor 1 (*RUNX1*)
RUNX3	864	Homo sapiens	Runt related transcription factor 3 (*RUNX3*)
CYP19A1	1588	Homo sapiens	Cytochrome P450 family 19 subfamily A member 1 (*CYP19A1*)
TP53BP1	7158	Homo sapiens	Tumor protein p53 binding protein 1 (*TP53BP1*)
MET	4233	Homo sapiens	MET proto-oncogene, receptor tyrosine kinase (*MET*)
SMAD4	4089	Homo sapiens	SMAD family member 4 (*SMAD4*)
FOXP1	27086	Homo sapiens	Forkhead box P1 (*FOXP1*)
NR1I2	8856	Homo sapiens	Nuclear receptor subfamily 1 group I member 2 (*NR1I2*)
FBXO32	114907	Homo sapiens	F-box protein 32 (*FBXO32*)
PTPN1	5770	Homo sapiens	Protein tyrosine phosphatase, non-receptor type 1 (*PTPN1*)
ABL1	25	Homo sapiens	ABL proto-oncogene 1, non-receptor tyrosine kinase (*ABL1*)
IER3	8870	Homo sapiens	Immediate early response 3 (*IER3*)
MSR1	4481	Homo sapiens	Macrophage scavenger receptor 1 (*MSR1*)
CCNE1	898	Homo sapiens	Cyclin E1 (*CCNE1*)
BARX1	56033	Homo sapiens	BARX homeobox 1 (*BARX1*)
CASP9	842	Homo sapiens	Caspase 9 (*CASP9*)
CASP7	840	Homo sapiens	Caspase 7 (*CASP7*)
HMOX1	3162	Homo sapiens	Heme oxygenase 1 (*HMOX1*)
CASP8	841	Homo sapiens	Caspase 8 (*CASP8*)
NOS3	4846	Homo sapiens	Nitric oxide synthase 3 (*NOS3*)
MYB	4602	Homo sapiens	MYB proto-oncogene, transcription factor (*MYB*)
MYC	4609	Homo sapiens	v-myc avian myelocytomatosis viral oncogene homolog (*MYC*)
TERT	7015	Homo sapiens	Telomerase reverse transcriptase (*TERT*)
TP53	7157	Homo sapiens	Tumor protein p53 (*TP53*)
PRKCI	5584	Homo sapiens	Protein kinase C iota (*PRKCI*)
CDK6	1021	Homo sapiens	Cyclin dependent kinase 6 (*CDK6*)
PADI4	23569	Homo sapiens	Peptidyl arginine deiminase 4 (*PADI4*)
MBNL1	4154	Homo sapiens	Muscleblind like splicing regulator 1 (*MBNL1*)
CDK4	1019	Homo sapiens	Cyclin dependent kinase 4 (*CDK4*)
MMP14	4323	Homo sapiens	Matrix metallopeptidase 14 (*MMP14*)
MMP12	4321	Homo sapiens	Matrix metallopeptidase 12 (*MMP12*)

Table 1 (*continued*)

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Gene abbreviations	Gene ID	Species	Gene name
TNFRSF10A	8797	Homo sapiens	TNF receptor superfamily member 10a (*TNFRSF10A*)
VSIG10L	147645	Homo sapiens	V-set and immunoglobulin domain containing 10 like (*VSIG10L*)
Myo9B	4650	Homo sapiens	Myosin IXB (*MYO9B*)
NCOA3	8202	Homo sapiens	Nuclear receptor coactivator 3 (*NCOA3*)
TARP	445347	Homo sapiens	TCR gamma alternate reading frame protein (*TARP*)
MDM2	4193	Homo sapiens	MDM2 proto-oncogene (*MDM2*)
GHRL	51738	Homo sapiens	Ghrelin and obestatin prepropeptide (*GHRL*)
JMJD1C	221037	Homo sapiens	Jumonji domain containing 1C (*JMJD1C*)
CHFR	55743	Homo sapiens	Checkpoint with forkhead and ring finger domains (*CHFR*)
GSTP1	2950	Homo sapiens	Glutathione S-transferase pi 1 (*GSTP1*)
DCC	1630	Homo sapiens	DCC netrin 1 receptor (*DCC*)
FKBP5	2289	Homo sapiens	FK506 binding protein 5 (*FKBP5*)
MGMT	4255	Homo sapiens	O-6-methylguanine-DNA methyltransferase (*MGMT*)
CDH1	999	Homo sapiens	Cadherin 1 (*CDH1*)
CDH3	1001	Homo sapiens	Cadherin 3 (*CDH3*)
IGF1R	3480	Homo sapiens	Insulin like growth factor 1 receptor (*IGF1R*)
TNFRSF1A	7132	Homo sapiens	TNF receptor superfamily member 1A (*TNFRSF1A*)
MTHFR	4524	Homo sapiens	Methylenetetrahydrofolate reductase (*MTHFR*)
MDC1	9656	Homo sapiens	Mediator of DNA damage checkpoint 1 (*MDC1*)
BCL2	596	Homo sapiens	BCL2, apoptosis regulator (*BCL2*)
TGM2	7052	Homo sapiens	Transglutaminase 2 (*TGM2*)
MTMR9	66036	Homo sapiens	Myotubularin related protein 9 (*MTMR9*)
ERCC1	2067	Homo sapiens	ERCC excision repair 1, endonuclease non-catalytic subunit (*ERCC1*)
APC	324	Homo sapiens	APC, WNT signaling pathway regulator (*APC*)
ERCC2	2068	Homo sapiens	ERCC excision repair 2, TFIIH core complex helicase subunit (*ERCC2*)
FLT1	2321	Homo sapiens	fms related tyrosine kinase 1 (*FLT1*)
GMDS	2762	Homo sapiens	GDP-mannose 4,6-dehydratase (*GMDS*)
VHL	7428	Homo sapiens	Von Hippel-Lindau tumor suppressor (*VHL*)
KLK3	354	Homo sapiens	Kallikrein related peptidase 3 (*KLK3*)
TBX5	6910	Homo sapiens	T-box 5 (*TBX5*)
IGF1	3479	Homo sapiens	Insulin like growth factor 1 (*IGF1*)
IGF2	3481	Homo sapiens	Insulin like growth factor 2 (*IGF2*)
BNC2	54796	Homo sapiens	Basonuclin 2 (*BNC2*)
PERP	64065	Homo sapiens	PERP, TP53 apoptosis effector (*PERP*)

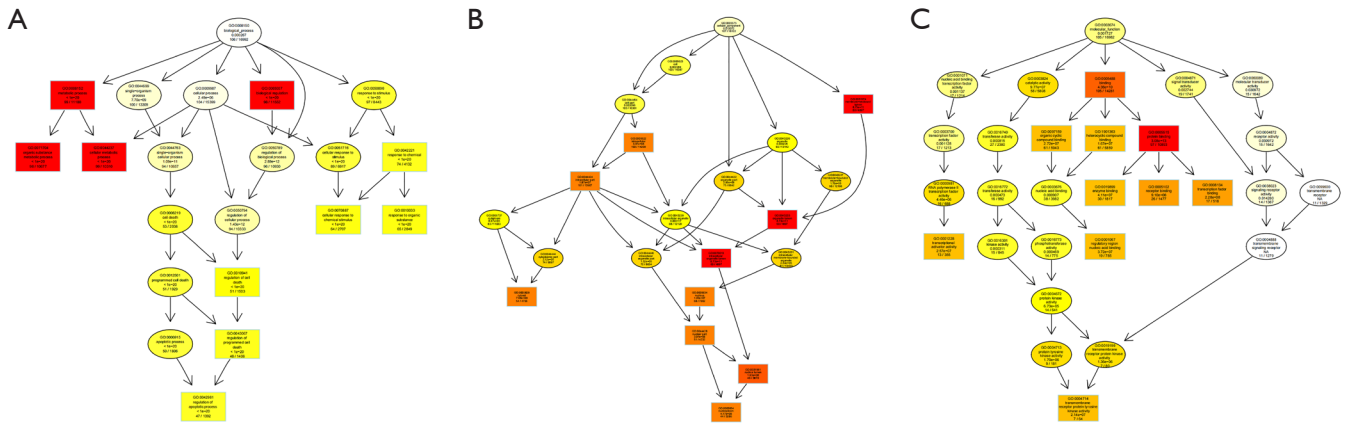

Figure 1 DAG of BP, CC, and MF. The branches represent the inclusion of GO terms. Top 10 GO enrichment terms in the rectangle and other related GO terms in the circle are shown by inclusion relationship, and more red indicates more significance. The defined function range becomes smaller from top to bottom. DAG, directed acyclic graph; BP, biological process; CC, cellular component; MF, molecular function; GO, Gene Ontology.

may be involved in drug metabolism, such as glutathione S-transferase mu 1 (*GSTM1*), glutathione S-transferase mu 3 (*GSTM3*), and cytochrome P450 family 2 subfamily E member 1 (*CYP2E1*).

GO enrichment analysis of EA

Functional enrichment analysis revealed a more specific function spectrum of these genes. Among the GO terms overrepresented in candidate genes were those associated with regulation and metabolic processes. In the BP category, terms directly associated with regulation and metabolic processes were identified, including organic substance metabolic process [Benjamini-Hochberg-corrected P (PBH) =1E-30], metabolic process (PBH =1E-30), cellular metabolic process (PBH =1E-30), and biological regulation (PBH =1E-30). Similarly, in the MF category, terms such as transcription factor binding (PBH =2.278E-08), receptor binding (PBH =9.10E-08), and protein binding (PBH =3.08E-13) were significantly enriched. In the CC category, the terms organelle lumen (PBH =8.73E-11), membrane-enclosed lumen (PBH =8.73E-11), and intracellular organelle lumen (PBH =8.73E-11) were extracted.

Enrichment of certain GO terms could be due to the contribution of other GO terms enriched at a lower hierarchy. Therefore, DAG was used to depict the results of GO enrichment analysis and how these affected other GO terms through upper hierarchies. For example, a part of the DAG representing BP (*Figure 1A*) was related with the GO term "biological regulation" in the rectangle. Surprisingly, this GO term was enriched at a very low FDR (<1e-20), and some other GO terms at lower hierarchies such as "regulation of biological process" and "regulation of cellular process" in the circle were enriched as a result. At the same, CC (*Figure 1B*) was associated with "intracellular", and the lower terms such as "intracellular organelle" and "intracellular organelle part" were enriched. Moreover, in MF (*Figure 1C*), the GO term "regulatory region nucleic acid binding" was enriched and so were its upper terms "nucleic acid binding" and "organic cyclic compound binding".

We used the ggplot2 package in Goseq software to depict the top 10 terms of BP, CC, and MF in graphs (*Figure 2A-2C*). For example, at the far left of the BP table, there were 4 biggest points representing "organic substance metabolic process", "metabolic process", "cellular metabolic process", and "biological regulation" with the P value less than 1e-20 and count of EA genes more than 90. Interestingly, the most significant dot here was also the most red in DAG. The same results could be observed in CC and MF.

Pathway enrichment analysis of EA

Detection of the biological pathways enriched in the candidate genes may provide important information about the pathogenic molecular mechanism underlying EA. Through KEGG analysis, we found 177 significant enrichment pathways and extracted 19 most significant

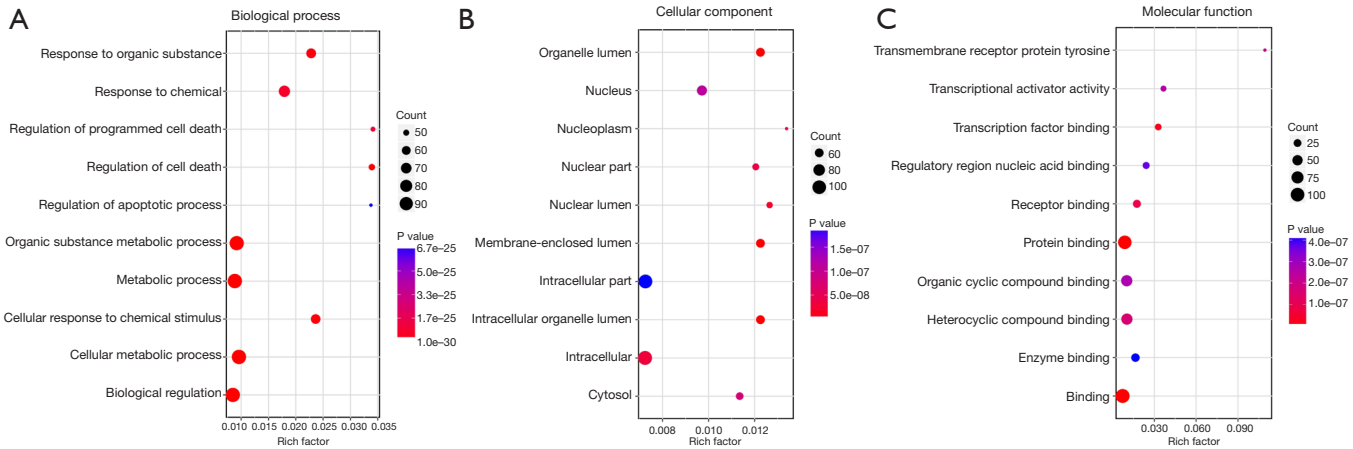

Figure 2 Dot plot graph of BP, CC, MF, and KEGG. The graph shows the richness factor and P value for the top 10 GO terms. The size of the solid dot indicates the count of EA-related genes in this term. BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; EA, esophageal adenocarcinoma.

enrichment pathways (FDR ≤0.05) (*Table 2*). Among them, several pathways related to cancer, including bladder cancer (PBH =1.702E-6), prostate cancer (PBH =8.588E-5), pancreatic cancer (PBH =1.741E-4), chronic myeloid leukemia (PBH =1.079E-3), and small cell lung cancer (PBH =1.017E-2). In addition, drug reaction and metabolism-related processes were identified, such as platinum drug resistance (PBH =1.371E-4) and microRNAs in cancer (PBH =0.030). Further, pathways associated with cell growth and survival, including the p53 signaling pathway (PBH =0.003) and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance (PBH =0.002), were also detected. The KEGG results in table 2 showed that "pathways in cancer" had an EA gene count of more than 30.

Crosstalk among significantly enriched pathways

To perform further analysis of the pathways and how they interact with each other, we implemented a pathway crosstalk analysis for the 19 enriched pathways. The screening conditions included more than 5 candidate genes per pathway and at least 3 genes for each pathway shared with 1 or more other pathways. Coincidentally, the 19 pathways met our screening criteria for crosstalk analysis. All of the 161 pathway pairs (edges) among the 19 pathways, which were ranked according to the average JC and OC scores, were used to construct the pathway crosstalk network. Based on the crosstalk, these pathways could be probably divided into 2 major modules, each of which contained pathways that had more crosstalk with each other compared with other pathways and were likely related to the similar biological processes or etiology (*Figure 3*). The first module included a variety of cancers, metabolism-related processes, and drug reaction pathways, such as bladder cancer, prostate cancer, pancreatic cancer, non-small cell lung cancer, endocrine resistance, microRNAs in cancer, proteoglycans in cancer, and platinum drug resistance. In the other module, cell transcriptional regulation predominated, including endometrial cancer, chronic myeloid leukemia, p53 signaling, HIF-1 signaling, and central carbon metabolism in cancer. As the above results indicated, pathway crosstalk analysis could provide important insight into the understanding of oncogenic mechanisms.

EA-specific protein network

To distill insight into the potential pathological protein network of EA, we constructed a subnetwork for EA from the human PPI network with the Klein-Ravi algorithm. Typically, this algorithm will connect as many input nodes as possible (genes included in EA genes) with the minimum number of interlinking nodes. As shown in *Figure 4*, the protein network of EA contained 116 nodes and 284 edges. Of the 109 EA genes, 104 were included in the EA-specific network, which accounted for about 95.4% of the EA genes and 89.7% of the genes in the EA-specific network, indicating a high coverage of EA genes in the subnetwork. In addition to EA genes, a further 12 genes were contained in the EA-specific network (*Table 3*). In view of the close

Table 2 Pathways enriched in the EA-related genes (top 19 pathways)

Pathway name	Database	ID	Input number	Background number	P value	Corrected P value	Gene ID	Gene name
Bladder cancer	KEGG PATHWAY	hsa05219	13	41	9.62E-09	1.70E-06	4312|4313|7157|999|1029|4193|1956|3845|1019|7422|4609|2064|1950	*MMP1|MMP2|TP53|CDH1|CDKN2A|MDM2|EGFR|KRAS|CDK4|VEGFA|MYC|ERBB2|EGF*
Prostate cancer	KEGG PATHWAY	hsa05215	15	89	9.70E-07	8.59E-05	842|596|7157|3480|2064|3479|5290|4193|898|354|1956|3845|1950|2263|2950	*CASP9|BCL2|TP53|IGF1R|ERBB2|IGF1|PIK3CA|MDM2|CCNE1|KLK3|EGFR|KRAS|EGF|FGFR2|GSTP1*
Melanoma	KEGG PATHWAY	hsa05218	13	71	2.27E-06	0.000134	1021|7157|3480|1029|3479|5290|4193|999|1956|3845|1019|4233|1950	*CDK6|TP53|IGF1R|CDKN2A|IGF1|PIK3CA|MDM2|CDH1|EGFR|KRAS|CDK4|MET|EGF*
Pathways in cancer	KEGG PATHWAY	hsa05200	33	397	3.02E-06	0.000134	596|4089|5290|5743|1630|25|7157|4233|3479|1019|324|2263|7474|4312|4313|898|3480|999|4193|1956|1950|4609|2950|1021|842|841|861|1029|354|3845|7422|2064|7428	*BCL2|SMAD4|PIK3CA|PTGS2|DCC|ABL1|TP53|MET|IGF1|CDK4|APC|FGFR2|WNT5A|MMP1|MMP2|CCNE1|IGF1R|CDH1|MDM2|EGFR|EGF|MYC|GSTP1|CDK6|CASP9|CASP8|RUNX1|CDKN2A|KLK3|KRAS|VEGFA|ERBB2|VHL*
Platinum drug resistance	KEGG PATHWAY	hsa01524	13	75	3.87E-06	0.000137	842|841|596|7157|2064|1029|4193|5290|2947|2944|2950|2952|2067	*CASP9|CASP8|BCL2|TP53|ERBB2|CDKN2A|MDM2|PIK3CA|GSTM3|GSTM1|GSTP1|GSTT1|ERCC1*
Pancreatic cancer	KEGG PATHWAY	hsa05212	12	66	5.90E-06	0.000174	1021|842|7157|4089|1029|7422|5290|1956|3845|1019|2064|1950	*CDK6|CASP9|TP53|SMAD4|CDKN2A|VEGFA|PIK3CA|EGFR|KRAS|CDK4|ERBB2|EGF*
Non-small cell lung cancer	KEGG PATHWAY	hsa05223	11	56	7.55E-06	0.000191	1021|842|2272|7157|1029|5290|1956|3845|1019|2064|1950	*CDK6|CASP9|FHIT|TP53|CDKN2A|PIK3CA|EGFR|KRAS|CDK4|ERBB2|EGF*
Endometrial cancer	KEGG PATHWAY	hsa05213	10	52	2.30E-05	0.000508	842|7157|999|5290|1956|3845|1950|324|4609|2064	*CASP9|TP53|CDH1|PIK3CA|EGFR|KRAS|EGF|APC|MYC|ERBB2*
Glioma	KEGG PATHWAY	hsa05214	11	65	2.60E-05	0.000511	1021|7157|3480|1029|3479|5290|4193|1956|3845|1019|1950	*CDK6|TP53|IGF1R|CDKN2A|IGF1|PIK3CA|MDM2|EGFR|KRAS|CDK4|EGF*
Endocrine resistance	KEGG PATHWAY	hsa01522	13	97	4.52E-05	0.000801	4313|8202|596|7157|3480|1029|3479|5290|4193|1956|3845|1019|2064	*MMP2|NCOA3|BCL2|TP53|IGF1R|CDKN2A|IGF1|PIK3CA|MDM2|EGFR|KRAS|CDK4|ERBB2*
Chronic myeloid leukemia	KEGG PATHWAY	hsa05220	11	73	6.71E-05	0.001079	25|1021|7157|4089|1029|4193|5290|861|3845|1019|4609	*ABL1|CDK6|TP53|SMAD4|CDKN2A|MDM2|PIK3CA|RUNX1|KRAS|CDK4|MYC*
EGFR tyrosine kinase inhibitor resistance	KEGG PATHWAY	hsa01521	11	81	0.000154	0.002278	596|3480|3479|5290|2064|1956|3845|1950|2263|4233|7422	*BCL2|IGF1R|IGF1|PIK3CA|ERBB2|EGFR|KRAS|EGF|FGFR2|MET|VEGFA*
p53 signaling pathway	KEGG PATHWAY	hsa04115	10	69	0.00019	0.002585	1021|842|841|7157|1029|3479|4193|64065|898|1019	*CDK6|CASP9|CASP8|TP53|CDKN2A|IGF1|MDM2|PERP|CCNE1|CDK4*

Table 2 (*continued*)

Table 2 (*continued*)

Pathway name	Database	ID	Input number	Background number	P value	Corrected P value	Gene ID	Gene name
HIF-1 signaling pathway	KEGG PATHWAY	hsa04066	12	103	0.000289	0.003654	2321|596|3480|7422|3479|5290|2064|1956|1950|4846|3162|7428	*FLT1|BCL2|IGF1R|VEGFA|IGF1|PIK3CA|ERBB2|EGFR|EGF|NOS3|HMOX1|VHL*
Colorectal cancer	KEGG PATHWAY	hsa05210	9	62	0.00039	0.004601	1630|842|596|7157|4089|5290|3845|324|4609	*DCC|CASP9|BCL2|TP53|SMAD4|PIK3CA|KRAS|APC|MYC*
Small cell lung cancer	KEGG PATHWAY	hsa05222	10	86	0.00092	0.010174	1021|842|2272|7157|596|5290|898|5743|1019|4609	*CDK6|CASP9|FHIT|TP53|BCL2|PIK3CA|CCNE1|PTGS2|CDK4|MYC*
Central carbon metabolism in cancer	KEGG PATHWAY	hsa05230	8	67	0.002516	0.0262	7157|2064|5290|1956|3845|4609|2263|4233	*TP53|ERBB2|PIK3CA|EGFR|KRAS|MYC|FGFR2|MET*
MicroRNAs in cancer	KEGG PATHWAY	hsa05206	20	299	0.003053	0.030023	25|4170|596|7157|3162|2064|7078|1029|4193|5290|5743|898|1956|3845|7422|324|4609|1021|4233|27086	*ABL1|MCL1|BCL2|TP53|HMOX1|ERBB2|TIMP3|CDKN2A|MDM2|PIK3CA|PTGS2|CCNE1|EGFR|KRAS|VEGFA|APC|MYC|CDK6|MET|FOXP1*
Proteoglycans in cancer	KEGG PATHWAY	hsa05205	15	205	0.004502	0.041943	4313|7078|7157|3480|2064|4193|5290|3479|3481|1956|3845|7422|4609|7474|4233	*MMP2|TIMP3|TP53|IGF1R|ERBB2|MDM2|PIK3CA|IGF1|IGF2|EGFR|KRAS|VEGFA|MYC|WNT5A|MET*

interaction of these genes with reported EA-related genes, they may also participate in the pathological process of EA. Notably, many of the EA-related genes, including *TYMS*, *GSTO1*, and *BMP7*, have been reported in previous studies (10-12). While some of these genes have not been shown to directly participate in the pathophysiology of EA, some of the genes linked to them or other member genotypes of the same protein family have been found to be involved in these reactions.

Discussion

Recently, considerable progress has been made in the study of the molecular mechanisms of EA. Advances in technology have allowed us to identify more and more genes and proteins associated with this disease on a larger scale. While a large number of studies have reported on the pathogenesis genes of EA, especially in the progression of Barrett's esophagus to EA, in-depth analysis of the biochemical processes related to the pathogenesis of EA at the molecular level is still limited. Besides, there is no literature to summarize and analyze the relationship between these genes and EA. As a result, a systematic analysis of EA-related genes based on pathways and networks would provide a valuable resource for analyzing gene function, biochemical pathways, and subnetworks of EA (13). In this study, we pooled and managed data from a number of studies on human genes associated with EA to provide a comprehensive analysis of EA-related biochemical processes and their interrelations.

Functional enrichment analysis has brought the specific biological processes involved in EA genes to light. Our results showed that genes involved in EA might play an important role in metabolic processes, cell growth and survival, and drug reactions. The terms cellular metabolic process, organic substance metabolic process, biological regulation, regulation of cell death, regulation of apoptotic process, and response to stimulus were significantly enriched in EA genes, suggesting they play an important role in the pathogenesis of EA.

Pathway analysis showed that various cancer pathways were enriched in EA genes, such as bladder cancer, prostate cancer, pancreatic cancer, and so forth. This might be due to the regulation of the same genes among various cancers and also confirmed that the occurrence of cancer is not a single molecular event but a multistep process. Meanwhile, it was noteworthy that almost all of these cancer pathways contained *TP53* and *PIK3CA*. As a well-known tumor

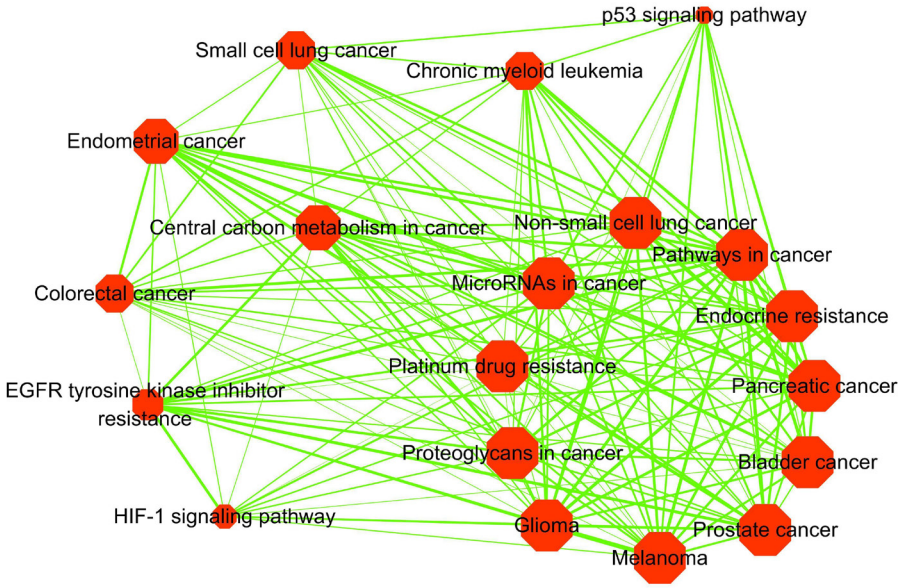

Figure 3 Pathway crosstalk among EA genes-enriched pathways. Crosstalk network indicating EA-overrepresented pathways. Nodes represent biological pathways and lines represent crosstalk among pathways. The width of the line is proportional to the crosstalk level of the given pathway pair, and the size of the node represents the degree of the pathways. EA, esophageal adenocarcinoma.

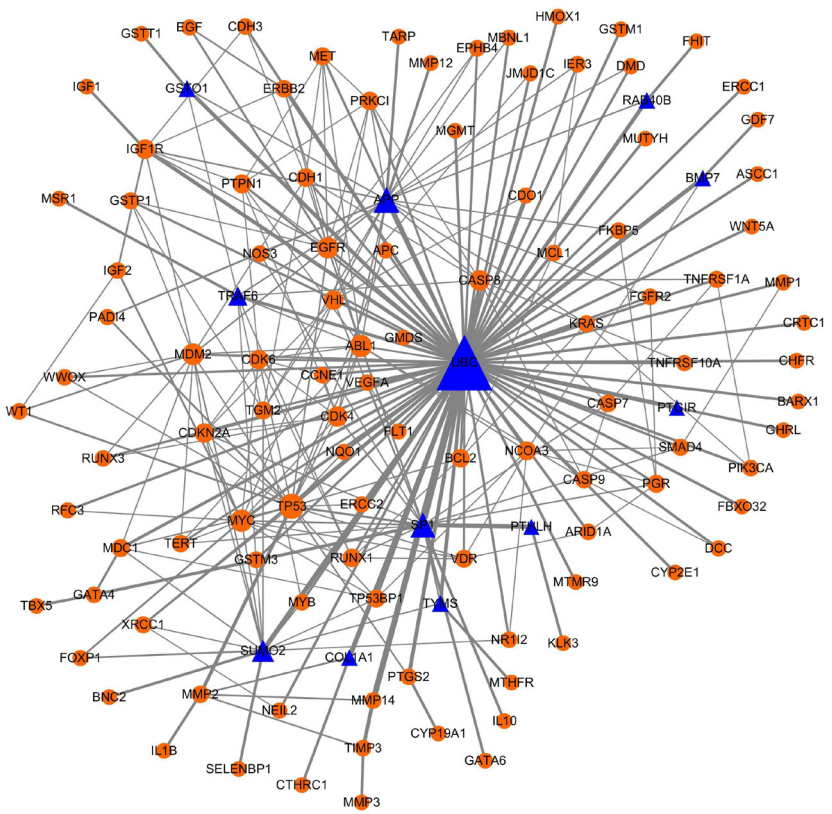

Figure 4 EA-specific network. The orange circular nodes represent EA genes and the blue triangle nodes are non-EA genes. Bigger size indicates higher degree. EA, esophageal adenocarcinoma.

Table 3 Twelve new genes associated with EA discovered by EA-specific network

Gene abbreviations	Gene ID	Species	Gene name
PTHLH	5744	Homo sapiens	parathyroid hormone like hormone (*PTHLH*)
SUMO2	6613	Homo sapiens	small ubiquitin-like modifier 2 (*SUMO2*)
TYMS	7298	Homo sapiens	thymidylate synthetase (*TYMS*)
APP	351	Homo sapiens	amyloid beta precursor protein (*APP*)
PTGIR	5739	Homo sapiens	prostaglandin I2 (prostacyclin) receptor (IP) (*PTGIR*)
SP1	6667	Homo sapiens	Sp1 transcription factor (*SP1*)
UBC	7316	Homo sapiens	ubiquitin C (*UBC*)
COL1A1	1277	Homo sapiens	collagen type I alpha 1 chain (*COL1A1*)
GSTO1	9446	Homo sapiens	glutathione S-transferase omega 1 (*GSTO1*)
TRAF6	7189	Homo sapiens	TNF receptor associated factor 6 (*TRAF6*)
BMP7	655	Homo sapiens	bone morphogenetic protein 7 (*BMP7*)
RAB40B	10966	Homo sapiens	RAB40B, member RAS oncogene family (*RAB40B*)

suppressor gene, *TP53*, also called *p53*, can induce cell cycle arrest to repair DNA damage or lead to apoptosis when growth arrest is not realized. However, after *p53* deletion or when mutated cells undergo DNA damage, the cells continue to increase, and hence the abnormality of DNA is transmitted to the progeny cells. More than 50% of human tumors carry mutations in the *p53* gene (14). *PIK3CA* encodes the p110α catalytic subunit of the class IA phosphatidylinositol 3-kinases (*PI3Ks*) and then initiates a complex signaling cascade reaction that can result in cell proliferation, survival, and regulation of motility, among others (15). At the same time, some pathways were associated with cell growth and survival. For example, as a transcription factor, *HIF-1* plays an important role in the formation of blood vessels and the proliferation of tumor cells (16). The generation of tumor blood vessels brings oxygen and nutrients to tumor cells, promotes the growth and proliferation of tumor cells, and is the basis for tumor cell invasion and metastasis. In addition, *EGFR* tyrosine kinase inhibitors block the abnormal signal transduction of *EGFR* and tyrosine kinase and induce apoptosis, thereby inhibiting the growth of tumor cells. *EGFR* tyrosine kinase inhibitors produce resistance via mechanisms such as *EGFR* mutations (17). Further, proteoglycans, as a sort of extracellular matrix in the tumor microenvironment, regulate the biological behavior of tumor cells via metabolic processes (18). These results suggested that the molecular mechanism of EA was rather intricate, involving many genes, pathways, and their interactions.

Importantly, in pathway crosstalk analysis, we established 2 major modules, of which 1 was mainly related to metabolic regulation and drug reactions. Among the pathways, we noted that many were tumor-associated pathways involved in metabolism. For example, the *MMP1* and *MMP2* genes in bladder cancer are members of the matrix metalloproteinase (*MMP*) gene family, which are involved in the breakdown of the extracellular matrix and play an important role in physiological processes, and thus their dysfunction is related to many diseases (including tumors) (19). In addition, the protein encoded by insulin like growth factor 1 (*IGF1*), which is found in prostate cancer, melanoma, and glioma, is similar to insulin in function and structure but has much higher growth promoting activity and is related to tumors (20). Similarly, platinum drug resistance, microRNAs, and endocrine resistance involved in metabolic regulation and drug reactions have been well studied (21). MicroRNAs directly or indirectly regulate several key enzymes and/or signaling hubs that result in the altering of metabolic pathways, leading to tumor progression and/or metastasis (22). Further, we noted that the 2 pathway modules were related to each other, and the genes in the pathways also interacted, suggesting that there was a transcription-metabolic regulatory network in the molecular mechanism of EA.

In addition, we extracted EA-specific protein networks based on the human PPI network. It was worth noting that

some genes did not belong to EA genes but were contained in the human PPI network which may be related to EA. For instance, *RAB40B* may be involved in tissue-supporting basement membrane or extracellular matrix destruction, and its encoded protein may regulate secretory vesicles, which may participate in tumor metastasis (23). *NF-κB* is activated in cells that become malignant tumors and cells that are recruited to and constitute the tumor microenvironment. *TRAF6* can serve as its activation molecule, leading to the expression of abnormal genes and malignancy (24). *TYMS* has recently been spotlighted as a target of cancer chemotherapeutics, particularly 5-FU, considering that it plays a role in DNA replication and repair (25). *SUMO2* plays a crucial role in nuclear transport, DNA replication and repair, mitosis, and signal transduction (26). As the results showed, network-based analysis could provide subnetworks for EA genes and also have the potential to detect promising related genes.

However, our study had some limitations. First, our analysis was based on reported EA-related genes from existing literature and as a result, the potential biases and deficiencies in the available reports inevitably affected our result. At the same time, there were few studies on the molecular mechanism of EA, and consequently, we might have omitted a number of important processes related to EA. Although the overall quality of PPI databases has been greatly improved, current technical limitations remain, and PPI data may also contain some false-positive results (27). With PPI data becoming more comprehensive and accurate, an EA-specific subnetwork will become more valuable.

Conclusions

We performed a comprehensive investigation into the pathways and molecular network of EA based on the EA genes. Integrating information from biological functions, biochemical pathways, and pathway crosstalk analyses, we found that biochemical processes and pathways associated with metabolic processes, biological regulation processes, and the signal transduction process played key roles in the molecular mechanism of EA. In addition, there were 2 interconnected pathway modules: 1 which included metabolic regulation and drug reactions, and the other transcriptional regulation. Furthermore, we extracted an EA-specific subnetwork and predicted some novel genes potentially bound up with EA. Our results provided critical information for further analysis and indicated that system level analysis was promising for exploring the molecular mechanisms of EA.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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