Atlantic Journal of Life Sciences
Volume 2025,Article ID 3, pages 11
https://doi.org/10.71005/b5jben88

Original Research Article

Exploration of Rosmarinic Acid as Anti-Esophageal Cancer Potential by use of Network Pharmacology and Molecular Docking Approaches

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Received 18 November 2024; Revised 19 January 2025; Accepted 27 January 2025; Published 8 February 2025

KEYWORDS

Rosmarinic acid
Esophageal cancer
Network pharmacology
Molecular docking
Signaling pathways

ABSTRACT

Esophageal cancer (EC) is a cancer with high lethality and poor prognosis, and it was responsible for the death of 0.54 million people in 2020. Its treatment is often challenged by the side effects of the drugs used and the development of resistance mechanisms by EC cells. In this study, the potential of rosmarinic acid (RA) to serve as a therapeutic regimen for the treatment of EC was evaluated using network pharmacology approach. Firstly, the putative targets of RA were identified using small molecule target prediction platforms, while the genes commonly dysregulated in EC were identified by microarray data analysis. Subsequently, common targets were identified and their interaction network was delineated using the STRING database, while the core targets of the network were identified using the CytoHubba plug-in of Cystoscope. Further analysis conducted included the gene ontology, pathways enrichment analysis, and molecular docking of RA with the core targets. The results of the study revealed that CDK2, CHEK1, ERBB2, GSK3β, HSP90AA1, MMP9, NFKβ1, and STAT1 to be the core targets via which RA might exhibit its anti-EC potential, and the molecular function mediated by these core targets include ATP binding and protein kinase activity. Critical pathways such as the interleukin-17 and PI3K-Akt signaling pathways which are commonly dysregulated in EC were also identified as the pathways RA may restore. Molecular docking simulation also revealed RA possess high binding affinities for the core targets. Ultimately, further confirmation of the anti-EC activity of RA should be conducted in experimental studies.

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1. Introduction

Esophageal cancer (EC), a form of malignancy that develops in the esophagus, is the sixth and seventh leading cause of cancer global mortality and morbidity, accounting /for 0.54 million deaths in 2020 (Chatterjee et al., 2023; Mazidimoradi et al., 2023). Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the major subtypes of EC, with each differing in the parts of the body in which they occur. Typically, ESCC affects the upper and middle portions of the esophagus, while EAC primarily arises in the lower esophagus, often at the junction with the stomach (Chatterjee et al., 2023). Interestingly, there exists another rare but aggressive subtype of esophageal cancer, namely, small cell esophageal carcinoma (SCC) (Jeene et al., 2019). It occurs in different parts of the body, including salivary glands, hypopharynx, esophagus, stomach, large and small intestine bladder, breast, and skin (Chatterjee et al., 2023). The treatment of EC often involves the use of chemotherapy, radiotherapy, targeted therapy, and immunotherapy, sometimes, these approaches are often together or in isolation (Obermannová et al., 2022; Chatterjee et al., 2023).

Chemotherapy is the first-line treatment for EC, with doxorubicin, cisplatin, and 5-fluorouracil among the most widely used chemotherapy drugs (He et al., 2021). However, their usage is often limited by the associated toxicity and drug resistance, hence, prompting the need for the development of newer therapeutics (Vrana et al., 2018). Over the years, medicinal plants have been recognized as a repertoire of compounds with numerous pharmacological properties, and have served as an attractive source for the identification of compounds that can be developed into therapeutic regimens for cancer treatment (Ogbodo et al., 2023). Exemplifying such compounds is RA, which has been reported to possess a panoply of pharmacological properties including anticancer, anti-inflammatory, antioxidant, and neuroprotective properties (Guan et al., 2022).

Notably, RA has shown cytotoxic and antiproliferative effects on triple-negative breast cancer cell lines and has been reported to inhibit microtubule affinity-regulating kinase 4, a protein linked to cancer progression (Anwar et al., 2020; Messeha et al., 2020). In a study by Rodríguez-Luna *et al*, they demonstrated that RA in combination with fucoxanthin mitigated UVB-induced apoptosis and inflammation via the downregulation of inflammasome components including NLRP3 and ASC, while upregulating NFR2 and HO-1 in human HaCaT keratinocytes, a UV radiation-induced skin cancer model (Rodríguez-Luna et al., 2019). Also, Ghiula *et al* reported RA induced apoptosis and autophagy in breast cancer cells, with the most pronounced effect noticed in estrogen-dependent MCF7 cells (Ghiulai et al., 2020). These findings suggest that RA can effectively target key mechanisms of cancer cell proliferation and survival. Given that esophageal cancer shares molecular characteristics such as the overexpression of proteins

that drive cell cycle progression and resistance to apoptosis with other aggressive cancers (The Cancer Genome Atlas Research Network, 2017), we hypothesized that RA's anticancer properties might extend to EC. Specifically, RA's ability to modulate kinase activity and affect cellular pathways associated with uncontrolled cell growth positioned it as a promising candidate for further exploration in EC treatment (Czerwińska and Radziejewska, 2024). However, studies evaluating its potential therapeutic effect on EC are lacking.

In this study, the potential of RA to serve as a viable compound for the treatment of EC via multitarget inhibition was evaluated using network pharmacology and molecular docking. It is worth noting that network pharmacology is an approach that allows for the exploration of the polypharmacological properties of compounds, providing a systems-level understanding of how a single compound can interact with multiple targets within cancer-related pathways (Liu et al., 2024). This approach is particularly relevant for complex diseases like EC, where multiple molecular pathways converge to drive tumor progression and resistance to treatment (Berger and Mardis, 2018). To complement this, molecular docking was employed to assess the binding affinity and interaction profile of RA, to gain structural insights into its interactions with critical cancer-associated targets . This combined approach enabled a comprehensive evaluation of RA's therapeutic potential against EC, especially in light of its previously documented inhibition of kinases and anti-inflammatory properties (Czerwińska and Radziejewska, 2024).

This study revealed that RA could exert therapeutic effects in EC by targeting key proteins including GSK3β, HSP90AA1, and NFκB. Furthermore, potential pathways and biological processes involved in RA's anticancer activity were also identified, offering novel insights into its mechanism and supporting its exploration as a multi-targeted therapeutic candidate for EC.

2. Materials and Methods

2.1. Compound retrieval and target prediction

The three-dimensional structure (3D) and the Simplified Molecular Input Line Entry System (SMILES) of RA were retrieved in structure data format (SDF) from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Kim et al., 2023). Subsequently, the putative targets of the compound were identified by utilizing small molecule target prediction servers including PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) (Wang et al., 2017), SwissTargetPrediction (Daina et al., 2019), Similarity Ensemble Approach (https://sea.bkslab.org/) (Keiser et al., 2007), and canSAR Black (https://cansarblack.icr.ac.uk/) (Mitsopoulos et al., 2021).

2.2. Gene expression analysis

The genes whose expression is dysregulated in the development and progression of esophagal cell adenocarcinoma, esophageal squamous cell carcinoma, and small cell esophageal carcinoma were identified by retrieving their respective microarray data from the Gene Expression Omnibus (GEO) database. Notably, microarray data on esophageal cell adenocarcinoma were retrieved using GSE26886 and GSE92396 (Wang et al., 2013), while microarray data on esophageal squamous cell carcinoma were retrieved using GSE20347 and GSE26886 (Hu et al., 2010; Wang et al., 2013). Furthermore, microarray data on small cell esophageal carcinoma was retrieved using GSE111044 (Liu et al., 2018). Following the retrieval of the gene expression data, GEO2R, a web-based R programming language program was employed for the identification of the differentially expressed genes (DEGs), with the settings kept default. The statistically significant DEGs were selected based on $p < 0.05$, |log FC|> 1 (Omoboyede et al., 2023). Subsequently, the DEGs in esophageal cell adenocarcinoma were identified by finding the intersecting DEGs of GSE26886 and GSE92396, while that of esophageal squamous cell carcinoma were identified by finding the intersecting DEGs of GSE20347 and GSE26886. Ultimately, the DEGs identified in esophageal cell adenocarcinoma, esophageal squamous cell carcinoma, and small cell esophageal carcinoma were unionized and taken for further analysis.

2.3. Identification of overlapping genes

The targets of RA that were also present in the DEGs from the previous step were identified by employing Venny 2.0 WebServer (Oliveros, 2007).

2.4. Protein-protein interaction network analysis and hub gene identification

The Search Tool for Retrieval of Interacting Genes (STRING) database v11.5 was employed to study the interaction between the identified overlapping genes. Notably, the organism of interest was selected as *homo sapiens* with a high confidence score of "0.70". Afterwards, the interaction network was visualized using the Cytoscape v3.10.2 software (Shannon et al., 2003), and the CytoHubba plug-in was thereafter utilized to identify the hub genes of the networks based on six different features of the plug-in, which include the degree of interactions, closeness, radiality, edge percolated component (EPC), maximal clique centrality (MCC), and maximum neighborhood component (MNC) (Chin et al., 2014; Olukunle et al., 2023).

2.5. Gene Set Enrichment Analysis

The hub genes identified from the previous step were functionally annotated using shinyGO V 0.76.2 (http://bioinformatics.sdstate.edu/go/) (Ge et al., 2020). The gene ontology was performed based on the biological process, cellular component, and molecular function, while the pathways were also identified via the Kyoto Encyclopaedia genes and genomes pathway. Ultimately, the built-in visualization tool of the Webserver was utilized for the plotting of the significant gene ontology and pathways.

2.6. Molecular Docking Simulation

The affinity of RA for the core targets of the PPI network was studied by molecular docking simulation. Notably, the 3D structures of the core targets including CDK2, CHEK1, ERRB2, GSK3β, HSP90AA1, MMP9, and STAT1 were retrieved from the Protein Databank (PDB) in PDB format, while NFKβ1 was predicted using the structure using the I-TASSER webserver (https://zhanggroup.org/I-TASSER/) (Yang and Zhang, 2015). The 3D structures of CDK2, CHEK1, ERRB2, GSK3β, HSP90AA1, MMP9, and STAT1 were retrieved using their respective PDB IDs which are 6GUE, 2HOG, 3PP0, 1Q41, 7UR3, 1GKC, and 1YVL (Rowsell et al., 2002; Bertrand et al., 2003; Mao et al., 2005; Fraley et al., 2006; Aertgeerts et al., 2011; Wood et al., 2019; Mishra et al., 2022), respectively, while the primary structure of NFKB1 was retrieved from the UniProt database with the UniProt ID "P19838" (Bateman et al., 2023). Following the retrieval of the structures, they were imported into the interface of Schrodinger Maestro 13.9 for preparatory procedure: using the LigPrep module, the structure of RA was prepared by utilizing the OPLS3e force field for structural optimization, while the protein structures were prepared using the protein preparation wizard. The protein preparatory steps included the addition of explicit hydrogens, water molecules deletion, het states generation at pH 7.0 ± 2.0 and PROPKA-based optimization of the protein structure, and the ultimate minimization of the structures using OPLS3e force field with the root mean square deviation (RMSD) value of heavy atoms set to 3.0 (Ogbodo et al., 2023). Thereafter, the receptor grid generation was used for the identification of the binding pockets of the proteins with co-crystalized ligands,

while the SiteMap tool was used for that of NFKβ1. Finally, the Glide tool of Maestro was utilized to perform the molecular docking simulation using the standard precision (SP) and extra precision (XP) algorithms of the same tool, using capecitabine as the standard drug.

3. Results

3.1. RA's potential therapeutic targets in esophageal cancer

A total of 420 unique targets were retrieved as putative targets of RA from the target prediction platforms. These targets include the caspase-6, peroxisome proliferator-activated receptor gamma, and insulin-like growth factor binding protein 3 among many others. Gene expression analysis revealed 1236 DEGs in esophageal cell adenocarcinoma, while 1116 and 6238 DEGs were identified in esophageal squamous cell carcinoma and small cell esophageal carcinoma, respectively. Specifically, 1484 and 5878 DEGs were identified from GSE92396 and GSE26886, respectively for esophageal cell adenocarcinoma, 1353 and 6013 DEGs were identified from GSE20347 and GSE26886, respectively, for esophageal squamous cell carcinoma, while all DEGs identified for small cell esophageal carcinoma were identified from GSE111044. Overall, the unification of the DEGs across the subtypes revealed 4790 unique DEGs. Ultimately, 130 targets were identified as probable targets of RA in esophageal cancer. Figures 1A and 1B depict the Venn diagram plots for the intersecting target identification and the structure of RA, respectively

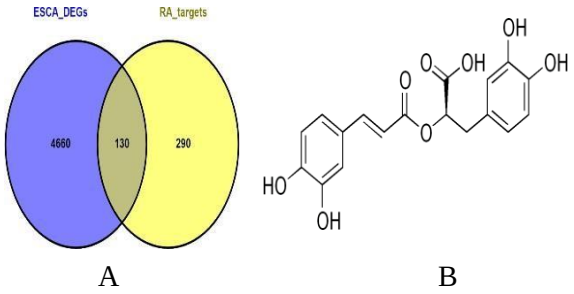


Figure 1: A) Venn diagram showing the identification of the targets of RA in esophageal cancer. **B)** The structure of RA. The circles colored blue and yellow shows the number of targets unique to esophageal cancer and RA, respectively, while the intersection shows the number of targets common to both.

3.2. Network analysis and core target prioritization for RA in esophageal cancer

The interaction network revealed the proteins that 108 out of the 130 targets interacted with each other in the biological system. Specifically, the network contained 108 nodes and 247 edges, a PPI enrichment value of < 1.0e-16, and average local clustering coefficient of 0.437. This shows that the interactions between the proteins are not random and indicate a connection in the biological milieu. The core targets of the PPI network included CDK2, CHEK1, ERBB2, GSK3β, HSP90AA1, MMP9, NFKβ1, and STAT1. Notably, these core targets were selected based on their consistent presence in at least four of the six algorithms that were utilized for core targets. These algorithms include the degree of interactions, closeness, radiality, edge percolated component (EPC), maximal clique centrality (MCC), and maximum neighborhood component (MNC). Figure 2 depicts the top ten core targets identified based on each of the algorithms.

3.3. Gene Set Enrichment Analysis

Gene ontology revealed the selected core targets were involved in different processes in the biological milieu: the biological processes mediated include phosphorylation, polysaccharide biosynthesis process, developmental growth, and response to oxygen-containing compounds, among others. Also, the molecular function includes ATP binding, kinase activity, protein serine/threonine/tyrosine kinase activity, and protein phosphatase binding, among others, while the cellular includes secretory granule, centrosome, insulin-like growth factor ternary complex, and cyclin A2-CDK2 complex. Furthermore, the targets are enriched in pathways including pathways in cancer, interleukin-17 and PI3K-Akt signaling pathways, growth hormone synthesis secretion and action, among others. Figure 3 depicts the visualization of the top twenty GO terms and pathways

3.4. Molecular Docking Simulation

As evident in Table 1, RA was found to possess high affinities for the selected targets as evident from its docking scores. Also, it interacts with amino acids present in the active sites of the targets. As evident in Table 1, RA exhibited high affinities for all the selected core targets in comparison with capecitabine, which is an approved chemotherapeutic drug. Of all the targets, RA was found to possess the lowest affinity for STAT1.

Table 1: The docking scores of RA against the selected core targets.

Protein	Compound	Docking Score (kcal/mol)
CDK2	RA	-6.896
	Seliciclib	-9.003
CHEK1	RA	-6.602
	Prexasertib	-8.925
ERBB2	RA	-5.061
	Lapatinib	-11.689
GSK3β	RA	-8.655
	CHIR-99021	-6.413
HSP90AA1	RA	-8.252
	Geldanamycin	-6.8
MMP9	RA	-8.055
	Marimastat	-9.148
NF-κB1	RA	-7.152
	BAY 11-7082	-3.240
STAT1	RA	-4.965
	Fludarabine	-5.060

The two-dimensional (2D) interaction of RA with the proteins are depicted in Figure 4. Evidently, RA interacted with several amino acid residues present in the active site of the proteins via numerous interactions including hydrogen bond, hydrophobic interactions, charged interactions, and polar interactions. Overall, RA was found to exhibit higher binding affinities for the targets compared to capecitabine.

4. Discussion

EC remains one of the deadliest cancers and it constitutes a major public health burden due to its high morbidity and mortality. Hence, the need to develop therapeutic modalities to combat its scourge is of high importance. This study aimed to evaluate the potential of RA as a candidate for EC treatment through multitarget inhibition using

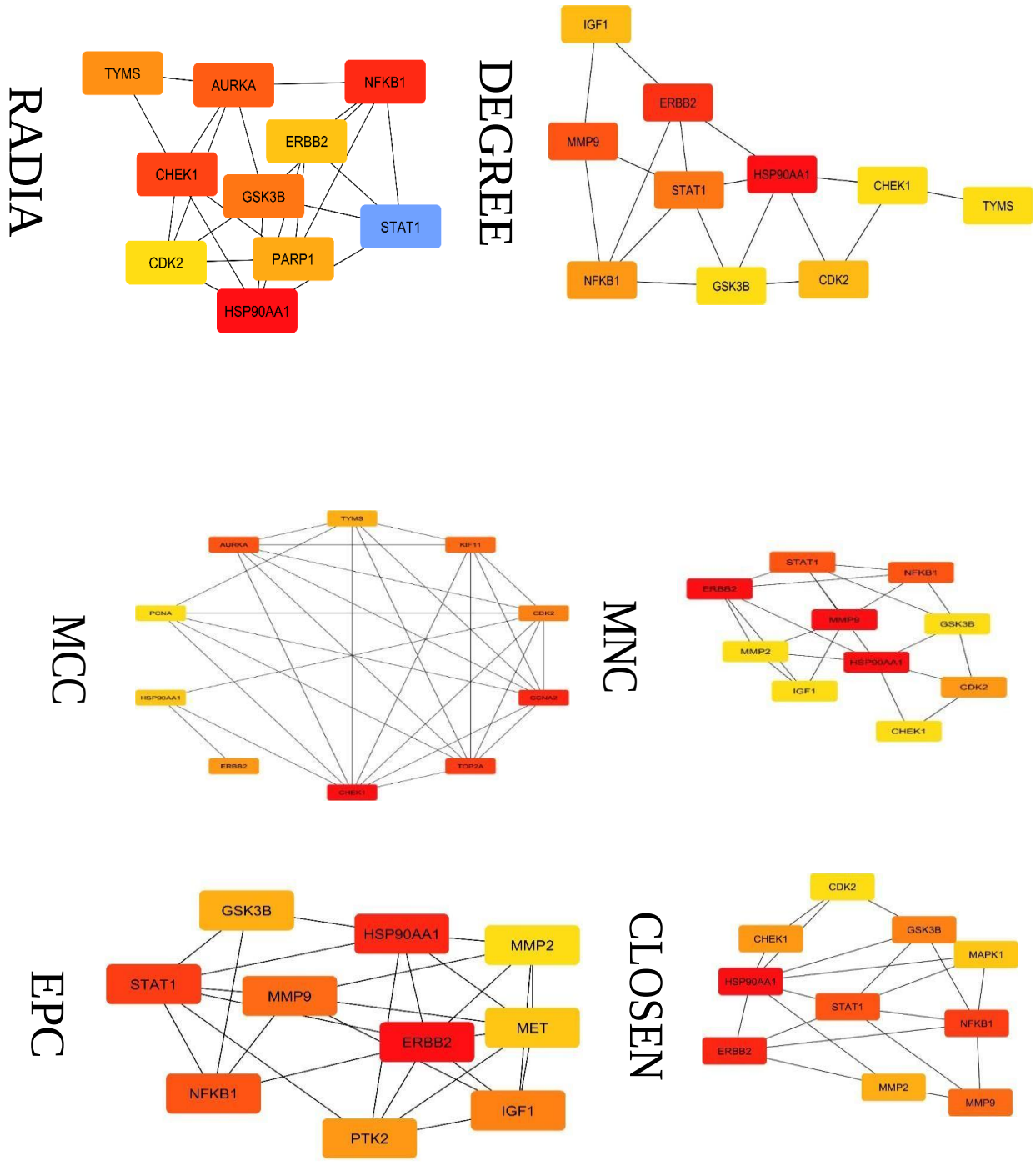


Figure 2: The top ten core targets of the PPI network identified by six different algorithms of CytoHubba Plug-in of Cytoscape

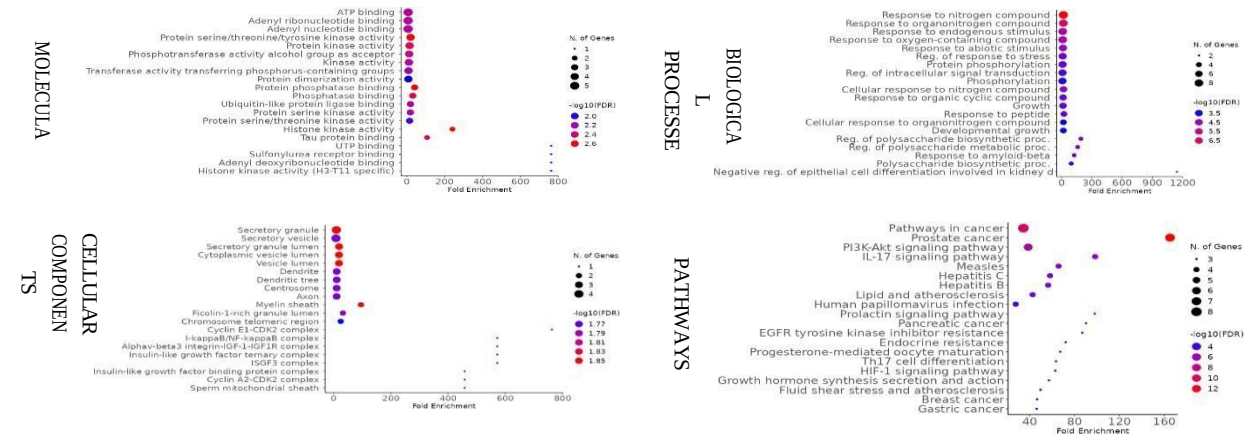
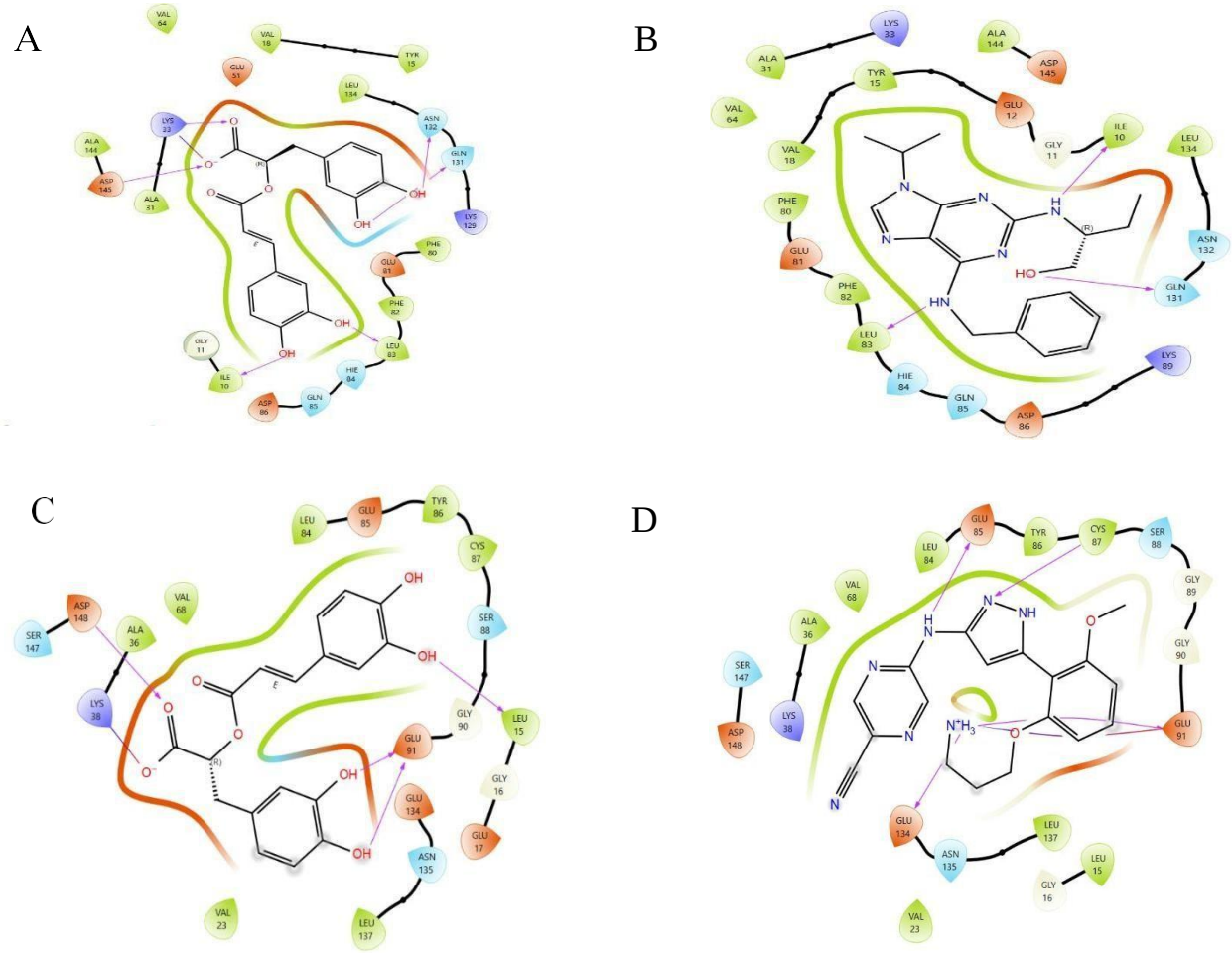
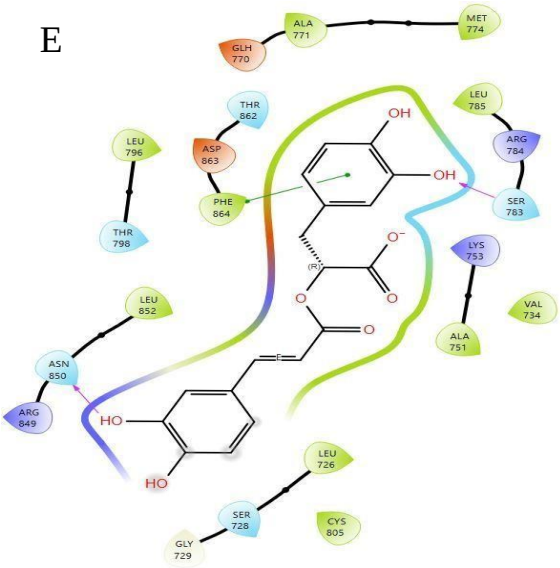


Figure 3: The top twenty gene ontology and pathways in which the selected core targets are enriched.



E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MET 774
GLH 770
THR 862
LEU 785
ARG 784
LEU 796
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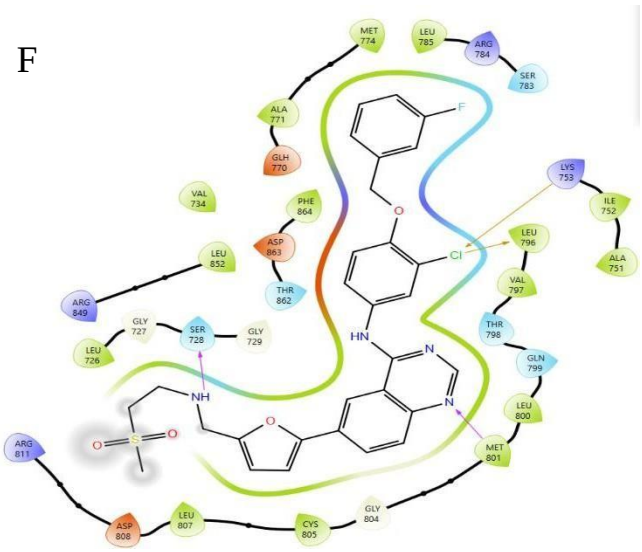
CYS 805
GLY 729

F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ARG 849
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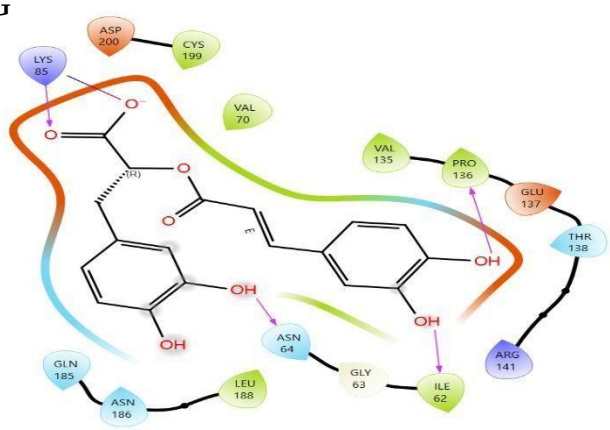
G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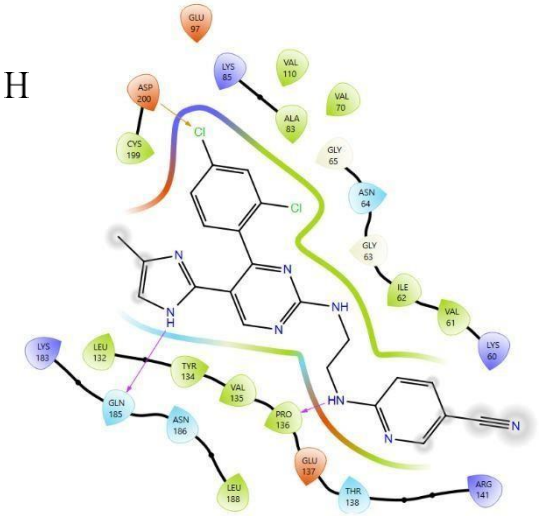
ASN 186
LEU 188

H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ILE 62
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THR 138
ARG 141

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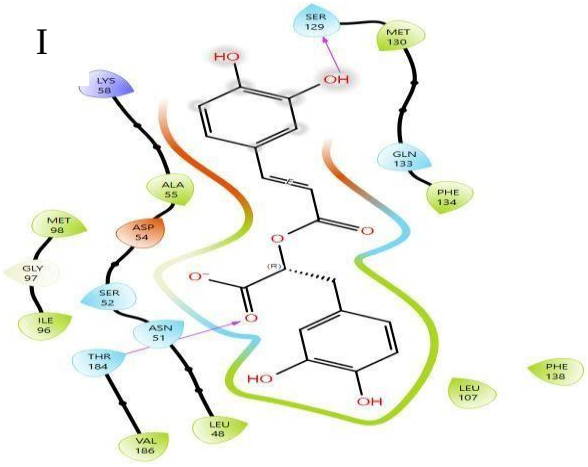
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VAL 186
LEU 48

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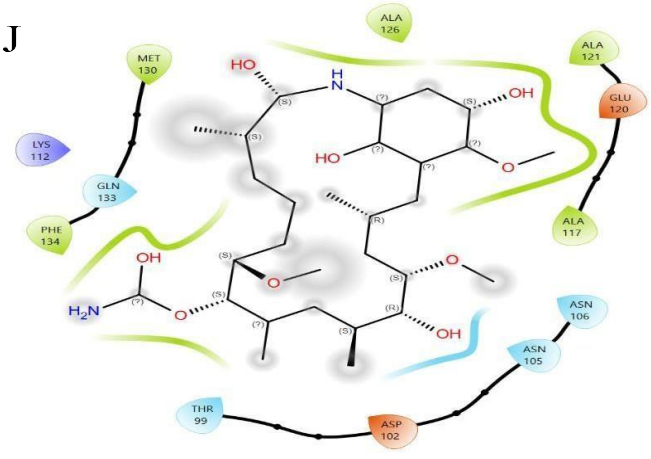
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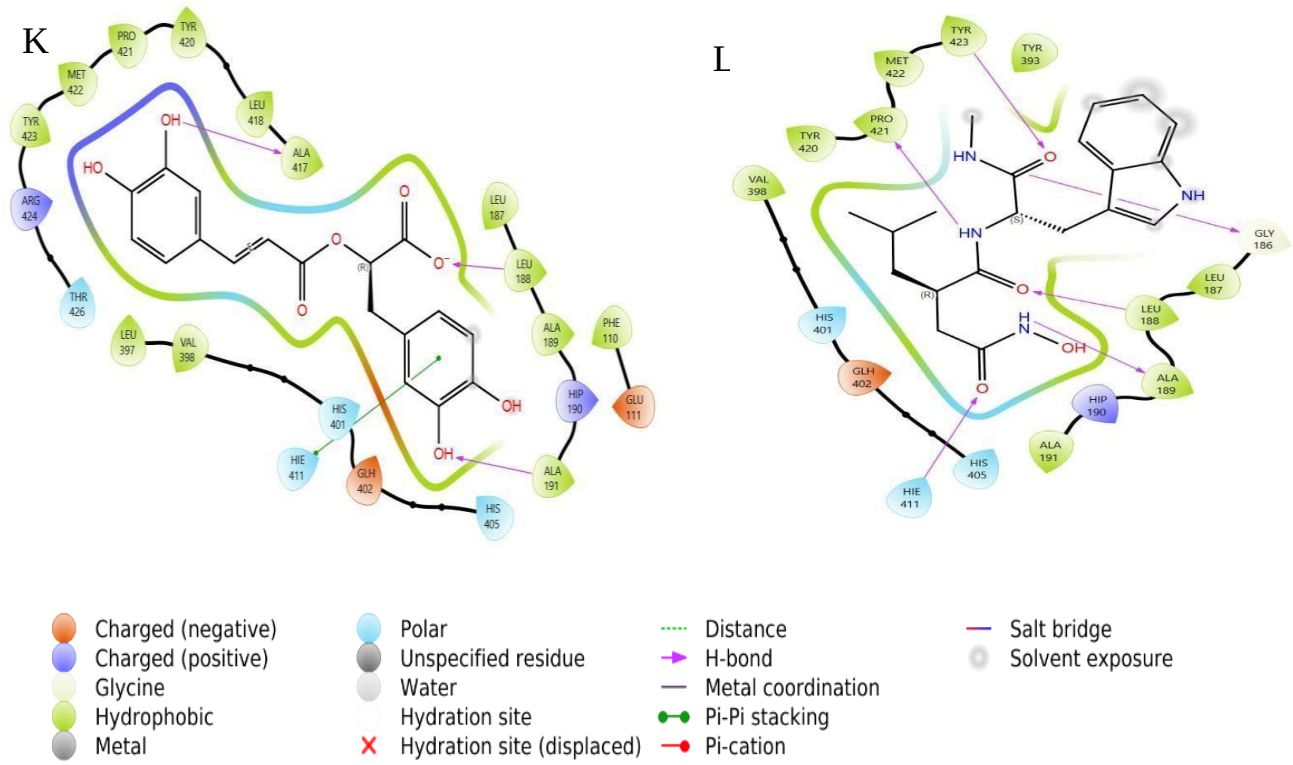

Figure 4: The 2D interactions of RA and capecitabine with the selected core targets. A) CDK2-rosmarinic acid; B) CDK2-seliciclib: C) CHEK1-rosmarinic acid; D) CHEK1- Prexasertib: E) ERBB2-rosmarinic acid; F) ERBB2- Lapatinib: G) GSK3β- rosmarinic acid; H) GSK3β-CHIR-99021: I) HSP90AA1-rosmarinic acid; J) HSP90AA1- Geldanamycin: K) MMP9-rosmarinic acid; L) MMP9- Marimastat: M) NFKβ1-rosmarinic acid; N) NFKβ1- BAY 11-7082; O) STAT1-rosmarinic acid; P) STAT1- Fludarabine.

network pharmacology and molecular docking.

RA has been reported to possess a plethora of pharmacological properties including anticancer, anti-inflammatory, and antioxidant properties (Guan et al., 2022). Hence, it is an attractive compound for anticancer drug discovery odysseys, particularly in diseases like EC, where effective multi-targeted therapies are lacking. Consequently, this study explored its potential to interact with critical mediators of EC development and progression, ultimately providing a comprehensive understanding of how it might contribute to anticancer efficacy.

Generally, the core targets with which RA is capable of interacting were found to be critical mediators of the development and progression of EC. CDK2, which plays a major role in cell cycle regulation, has been reported to be dysregulated in EC, and its inhibition has been reported to arrest the growth of EC (Chen et al., 2023). In this study, RA was found to be capable of interacting with the critical residues of the ATP binding site of CDK2: these residues include LYS33, GLU51, PHE80, LEU83, HIS84, GLN85, ASP86, and ASP145 (Li et al., 2015). It is worth noting that the inhibition of the critical residues of the ATP-binding site will result in the prevention of the binding of ATP and subsequent phosphorylation of its target proteins, hence, interrupting the downstream signaling pathways involved in cell proliferation and differentiation (Li et al., 2015). Interestingly, a study by Zhou *et al* found that benzydamine-mediated inhibition of the activity of CDK2 decreased the phosphorylation of MCM2, retinoblastoma, and c-MYC proteins (Zhou et al., 2023). Hence, RA might exhibit anti-EC activity by modulating CDK2 activity as previously reported in a study by Zhou *et al* based on their results on RA's anti-pancreatic cancer activity (Zhou et al., 2022).

Similarly, the inhibition of GSK3β in ESCC has been reported to induce cell cycle arrest at the G0/G1- and G2/M-phases, downregulate the expression of cyclin D1 and CDK4, and upregulate the expression of cyclin B1 (Bolidong et al., 2020). RA was found to interact with GLN-185, LYS-183, ILE-62, ASN-186, ARG-141, VAL-135, and ASP-133 which have been observed to be conserved across inhibitors of GSK3β which have been reported in some studies (Smith et al., 2001; Witherington et al., 2003).

Further analysis of the potentials of RA reveals that it might be involved in the simultaneous activation and inhibition of some proteins in EC: STAT1, which plays tumor suppressive roles and apoptotic roles in cancer, has been reported to be frequently downregulated in ESCC, and its downregulation is correlated with poor prognosis (Zhang et al., 2014). In the context of the current study, RA could potentially function in the enhancement of STAT1-dependent gene activation, which might contribute to the exertion of tumor-suppressive functions. Also, RA might exhibit the inhibition of MMP9 which has been reported to play a significant role in EC progression and metastasis. Specifically, MMP9 promotes tumor angiogenesis and hematogenous metastasis by degrading type IV

collagen and promoting VEGF expression (Wang et al., 2023). Different scaffolds of RA were found to interact with the amino acid residues of MMP9 via hydrogen bond formation with LEU188, ALA191, and ALA417, as well as hydrophobic interactions with PHE110, LEU187, LEU188, ALA189, ALA191, LEU397, VAL398, ALA417, LEU418, TYR420, PRO421, MET422, and TYR423. Interestingly, a study by Liu *et al*. also reported the interaction of the hit compounds of their study with some of the aforementioned amino acid residues (Liu et al., 2021). Other selected core targets also play a vital role in cancer progression: the upregulation of HSP90AA1 has been reported to reduce the sensitivity of osteosarcoma cells to chemotherapy, inhibit apoptosis, and promote autophagy, through the PI3K/Akt/mTOR pathway (Xiao et al., 2018). Interestingly, this pathway was identified via KEGG pathways enrichment analysis as one of the pathways via which RA could potentially mediate its therapeutic effect. Notably, the PI3K/Akt pathway promotes cell survival, proliferation, and metastasis by enhancing their motility and invasiveness (Luo et al., 2022). RA could function in the deactivation of the pathway via the modulation of the core targets. Another pathway via which RA could exert its therapeutic function is the IL-17 pathway, which has been reported to promote the invasiveness of EAC via its pro-inflammatory ability, which leads to the generation of reactive oxygen species and the expression of NF-κB1, which in turn mediate the activation of MMP-9 and MMP-9 (Liu et al., 2017). RA could potentially downregulate the expression of NF-κB1, whose hyperactivation correlates with the invasion of ESCC (Lehman et al., 2018). Interestingly, RA interacts with residues in the active site of the NF-κB1, including hydrogen bonds with ASP185, ARG230, and SER242, while also forming hydrophobic interactions with PHE227, LEU231, ALA238, ILE239, ILE252, TYR285, and VAL293. This phenomenon might prevent NF-κB1 from activating genes involved in biological processes including cell proliferation and survival, angiogenesis, and metastasis (Li et al., 2024).

Of all the aforementioned key targets, RA was found to have a higher binding affinity for NF-κB1, HSP90AA1, and GSK3β compared to standard compounds that have been well validated against them, suggesting that it will exert its major therapeutic effect via this route.

5. Conclusion

This study presents rosmarinic acid as a potential multi-targeted therapeutic candidate for esophageal cancer. Via its interaction with core proteins such as G CDK2, GSK3β, STAT1, MMP9, HSP90AA1, and NF-κB1, rosmarinic acid may inhibit mechanisms involved in esophageal cancer progression, including cell cycle regulation, angiogenesis, metastasis, and inflammatory signaling. Results from the pathway enrichment analyses further suggest that RA could disrupt the PI3K/Akt and IL-17 pathways, which are frequently dysregulated in EC. Also, molecular docking studies indicate rosmarinic acid's higher affinity for NF-κB1, HSP90AA1, and GSK3β compared to well-validated inhibitors, highlighting its potential as a leading candidate for further *in vitro* and *in vivo* validation. Overall, the findings of this study provide scientific rationale for future experimental studies exploring RA's anti-EC properties and contribute to the ongoing search for effective natural compounds in cancer therapeutics.

Conflict of interest

The authors declare no conflict of interest

Data Availability Statement

All data generated or analysed during this study are included in this published article

6. References

Aertgeerts, K., Skene, R., Yano, J., Sang, B.-C., Zou, H., Snell, G., et al. (2011). Structural Analysis of the Mechanism of Inhibition and Allosteric Activation of the Kinase Domain of HER2 Protein. *Journal of Biological Chemistry* 286, 18756–18765. doi: 10.1074/jbc.M110.206193

Anwar, S., Shamsi, A., Shahbaaz, M., Queen, A., Khan, P., Hasan, G. M., et al. (2020). Rosmarinic Acid Exhibits Anticancer Effects via MARK4 Inhibition. *Sci Rep* 10, 10300. doi: 10.1038/s41598-020-65648-z

Bateman, A., Martin, M.-J., Orchard, S., Magrane, M., Ahmad, S., Alpi, E., et al. (2023). UniProt: the Universal Protein Knowledgebase in 2023. *Nucleic Acids Research* 51, D523–D531. doi: 10.1093/nar/gkac1052

Berger, M. F., and Mardis, E. R. (2018). The emerging clinical relevance of genomics in cancer medicine. *Nature Reviews Clinical Oncology* 15, 353–365. doi: 10.1038/s41571-018-0002-6

Bertrand, J. A., Thieffine, S., Vulpetti, A., Cristiani, C., Valsasina, B., Knapp, S., et al. (2003). Structural Characterization of the GSK-3β Active Site Using Selective and Non-selective ATP-mimetic Inhibitors. *Journal of Molecular Biology* 333, 393–407. doi: 10.1016/j.jmb.2003.08.031

Bolidong, D., Domoto, T., Uehara, M., Sabit, H., Okumura, T., Endo, Y., et al. (2020). Potential therapeutic effect of targeting glycogen synthase kinase 3β in esophageal squamous cell carcinoma. *Sci Rep* 10, 11807. doi: 10.1038/s41598-020-68713-9

Chatterjee, D., Rahman, M. M., Saha, A. K., Siam, M. K. S., and Sharif Shohan, M. U. (2023). Transcriptomic analysis of esophageal cancer reveals hub genes and networks involved in cancer progression. *Computers in Biology and Medicine* 159, 106944. doi: 10.1016/j.compbiomed.2023.106944

Chen, Y., Dai, X., Chen, W., Qiao, Y., Bai, R., Duan, X., et al. (2023). Diosmetin suppresses the progression of ESCC by CDK2/Rb/E2F2/RRM2 pathway and synergies with cisplatin. *Oncogene* 42, 2278–2293. doi: 10.1038/s41388-023-02750-2

Chin, C.-H., Chen, S.-H., Wu, H.-H., Ho, C.-W., Ko, M.-T., and Lin, C.-Y. (2014). cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Systems Biology* 8, S11. doi: 10.1186/1752-0509-8-S4-S11

Czerwińska, K., and Radziejewska, I. (2024). Rosmarinic Acid: A Potential Therapeutic Agent in Gastrointestinal Cancer Management—A Review. *International Journal of Molecular Sciences* 25, 11704. doi: 10.3390/ijms252111704

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

Fraley, M. E., Steen, J. T., Brnardic, E. J., Arrington, K. L., Spencer, K. L., Hanney, B. A., et al. (2006). 3-(Indol-2-yl)indazoles as Chek1 kinase inhibitors: Optimization of potency and selectivity via substitution at C6. *Bioorganic & Medicinal Chemistry Letters* 16, 6049–6053. doi: 10.1016/j.bmcl.2006.08.118

Ge, S. X., Jung, D., and Yao, R. (2020). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics* 36, 2628–2629. doi: 10.1093/bioinformatics/btz931

Ghiulai, R., Avram, S., Stoian, D., Pavel, I. Z., Coricovac, D., Oprean, C., et al. (2020). Lemon Balm Extracts Prevent Breast Cancer Progression *In Vitro* and *In Ovo* on Chorioallantoic Membrane Assay. *Evidence-Based Complementary and Alternative Medicine* 2020, 6489159. doi: 10.1155/2020/6489159

Guan, H., Luo, W., Bao, B., Cao, Y., Cheng, F., Yu, S., et al. (2022). A Comprehensive Review of Rosmarinic Acid: From Phytochemistry to Pharmacology and Its New Insight. *Molecules* 27, 3292. doi: 10.3390/molecules27103292

He, S., Xu, J., Liu, X., and Zhen, Y. (2021). Advances and challenges in the treatment of esophageal cancer. *Acta Pharmaceutica Sinica B* 11, 3379–3392. doi: 10.1016/j.apsb.2021.03.008

Hu, N., Clifford, R. J., Yang, H. H., Wang, C., Goldstein, A. M., Ding, T., et al. (2010). Genome wide analysis of DNA copy number neutral loss of heterozygosity (CNNLOH) and its relation to gene expression in esophageal squamous cell carcinoma. *BMC Genomics* 11, 576. doi: 10.1186/1471-2164-11-576

Jeene, P. M., Geijsen, E. D., Muijs, C. T., Rozema, T., Aleman, B. M. P., Muller, K., et al. (2019). Small Cell Carcinoma of the Esophagus: A Nationwide Analysis of Treatment and Outcome at Patient Level in Locoregional Disease. *American Journal of Clinical Oncology* 42, 534–538. doi: 10.1097/COC.0000000000000546

Keiser, M. J., Roth, B. L., Armbruster, B. N., Ernsberger, P., Irwin, J. J., and Shoichet, B. K. (2007). Relating protein pharmacology by ligand chemistry. *Nat Biotechnol* 25, 197–206. doi: 10.1038/nbt1284

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., et al. (2023). PubChem 2023 update. *Nucleic Acids Research* 51, D1373–D1380. doi: 10.1093/nar/gkac956

Lehman, H. L., Kidacki, M., Warrick, J. I., and Stairs, D. B. (2018). NFkB hyperactivation causes invasion of esophageal squamous cell carcinoma with EGFR overexpression and p120-catenin down-regulation. *Oncotarget* 9, 11180–11196. doi: 10.18632/oncotarget.24358

Li, Y., Zhang, J., Gao, W., Zhang, L., Pan, Y., Zhang, S., et al. (2015). Insights on Structural Characteristics and Ligand Binding Mechanisms of CDK2. *IJMS* 16, 9314–9340. doi: 10.3390/ijms16059314

Li, Y., Zhao, B., Peng, J., Tang, H., Wang, S., Peng, S., et al. (2024). Inhibition of NF-κB signaling unveils novel strategies to overcome drug resistance in cancers. *Drug Resistance Updates* 73, 101042. doi: 10.1016/j.drup.2023.101042

Liu, D., Xu, X., Wen, J., Xie, L., Zhang, J., Shen, Y., et al. (2018). Integrated Genome-Wide Analysis of Gene Expression and DNA Copy Number Variations Highlights Stem Cell-Related Pathways in Small Cell Esophageal Carcinoma. *Stem Cells Int* 2018, 3481783. doi: 10.1155/2018/3481783

Liu, D., Zhang, R., Wu, J., Pu, Y., Yin, X., Cheng, Y., et al. (2017). Interleukin-17A promotes esophageal adenocarcinoma cell invasiveness through ROS-dependent, NF-κB-mediated MMP-2/9 activation. *Oncology Reports* 37, 1779–1785. doi: 10.3892/or.2017.5426

Liu, N., Wang, X., Wu, H., Lv, X., Xie, H., Guo, Z., et al. (2021). Computational study of effective matrix metalloproteinase 9 (MMP9) targeting natural inhibitors. *Aging* 13, 22867–22882. doi: 10.18632/aging.203581

Liu, Y., Li, X., Chen, C., Ding, N., Ma, S., and Yang, M. (2024). Exploration of compatibility rules and discovery of active ingredients in TCM formulas by network pharmacology. *Chinese Herbal Medicines*. doi: 10.1016/j.chmed.2023.09.008

Luo, Q., Du, R., Liu, W., Huang, G., Dong, Z., and Li, X. (2022). PI3K/Akt/mTOR Signaling Pathway: Role in Esophageal Squamous Cell Carcinoma, Regulatory Mechanisms and Opportunities for Targeted Therapy. *Front. Oncol.* 12, 852383. doi: 10.3389/fonc.2022.852383

Mao, X., Ren, Z., Parker, G. N., Sondermann, H., Pastorello, M. A., Wang, W., et al. (2005). Structural Bases of Unphosphorylated STAT1 Association and Receptor Binding. *Molecular Cell* 17, 761–771. doi: 10.1016/j.molcel.2005.02.021

Mazidimoradi, A., Ghavidel, F., Momenimovahed, Z., Allahqoli, L., and Salehiniya, H. (2023). Global incidence, mortality, and burden of esophageal cancer, and its correlation with SDI, metabolic risks, fasting plasma glucose, LDL cholesterol, and body mass index: An ecological study. *Health Science Reports* 6, e1342. doi: 10.1002/hsr2.1342

Messeha, S. S., Zarmouh, N. O., Asiri, A., and Soliman, K. F. A. (2020). Rosmarinic acid-induced apoptosis and cell cycle arrest in triple-negative breast cancer cells. *European Journal of Pharmacology* 885, 173419. doi: 10.1016/j.ejphar.2020.173419

Mishra, S. J., Reynolds, T. S., Merfeld, T., Balch, M., Peng, S., Deng, J., et al. (2022). Structure–Activity Relationship Study of Tertiary Alcohol Hsp90α-Selective Inhibitors with Novel Binding Mode. *ACS Med. Chem. Lett.* 13, 1870–1878. doi: 10.1021/acsmedchemlett.2c00327

Mitsopoulos, C., Di Micco, P., Fernandez, E. V., Dolciami, D., Holt, E., Mica, I. L., et al. (2021). canSAR: update to the cancer translational research and drug discovery knowledgebase. *Nucleic Acids Research* 49, D1074–D1082. doi: 10.1093/nar/gkaa1059

Obermannová, R., Alsina, M., Cervantes, A., Leong, T., Lordick, F., Nilsson, M., et al. (2022). Oesophageal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Annals of Oncology* 33, 992–1004. doi: 10.1016/j.annonc.2022.07.003

Ogbodo, U. C., Balogun, T. A., and Omoboyede, V. (2023). Integrated computational approach identifies potential inhibitors of ASK1-(JNK/P38) interaction signaling: new insights into cancer therapeutics. *Journal of Biomolecular Structure and Dynamics*, 1–14. doi: 10.1080/07391102.2023.2196699

Oliveros, J. C. (2007). VENNY. An interactive tool for comparing lists with Venn Diagrams. *http://bioinfogp. cnb. csic. es/tools/venny/index. html*.

Olukunle, O. F., Omoboyede, V., and Chukwuemeka, P. O. (2023). Network pharmacology and molecular docking-based identification of drug candidates and key targets of *Allium sativum* for colorectal cancer treatment. *Journal of Biomolecular Structure and Dynamics*, 1–14. doi: 10.1080/07391102.2023.2220823

Omoboyede, V., Ibrahim, O., Umar, H. I., Oke, G. A., Onile, O. S., and Chukwuemeka, P. O. (2023). Computer-aided analysis of quercetin mechanism of overcoming docetaxel resistance in docetaxel-resistant prostate cancer. *J Genet Eng Biotechnol* 21, 47. doi: 10.1186/s43141-023-00498-6

Rodríguez-Luna, A., Ávila-Román, J., Oliveira, H., Motilva, V., and Talero, E. (2019). Fucoxanthin and Rosmarinic Acid Combination Has Anti-Inflammatory Effects through Regulation of NLRP3 Inflammasome in UVB-Exposed HaCaT Keratinocytes. *Marine Drugs* 17, 451. doi: 10.3390/md17080451

Rowsell, S., Hawtin, P., Minshull, C. A., Jepson, H., Brockbank, S. M. V., Barratt, D. G., et al. (2002). Crystal Structure of Human MMP9 in Complex with a Reverse Hydroxamate Inhibitor. *Journal of Molecular Biology* 319, 173–181. doi: 10.1016/S0022-2836(02)00262-0

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research* 13, 2498–2504. doi: 10.1101/gr.1239303

Smith, D. G., Buffet, M., Fenwick, A. E., Haigh, D., Ife, R. J., Saunders, M., et al. (2001). 3-Anilino-4-arylmaleimides: potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorganic & Medicinal Chemistry Letters* 11, 635–639. doi: 10.1016/S0960-894X(00)00721-6

The Cancer Genome Atlas Research Network (2017). Integrated genomic characterization of oesophageal carcinoma. *Nature* 541, 169–175. doi: 10.1038/nature20805

Vrana, D., Hlavac, V., Brynychova, V., Vaclavikova, R., Neoral, C., Vrba, J., et al. (2018). ABC Transporters and Their Role in the Neoadjuvant Treatment of Esophageal Cancer. *Int J Mol Sci* 19, 868. doi: 10.3390/ijms19030868

Wang, Q., Ma, C., and Kemmner, W. (2013). Wdr66 is a novel marker for risk stratification and involved in epithelial-mesenchymal transition of esophageal squamous cell carcinoma. *BMC Cancer* 13, 137. doi: 10.1186/1471-2407-13-137

Wang, X., Shen, Y., Wang, S., Li, S., Zhang, W., Liu, X., et al. (2017). PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. *Nucleic Acids Research* 45, W356–W360. doi: 10.1093/nar/gkx374

Wang, Y., Yang, W., Wang, Q., and Zhou, Y. (2023). Mechanisms of esophageal cancer metastasis and treatment progress. *Front. Immunol.* 14, 1206504. doi: 10.3389/fimmu.2023.1206504

Witherington, J., Bordas, V., Gaiba, A., Naylor, A., Rawlings, A. D., Slingsby, B. P., et al. (2003). 6-Heteroaryl-pyrazolo[3,4-b]pyridines: potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorganic & Medicinal Chemistry Letters* 13, 3059–3062. doi: 10.1016/S0960-894X(03)00646-2

Wood, D. J., Korolchuk, S., Tatum, N. J., Wang, L.-Z., Endicott, J. A., Noble, M. E. M., et al. (2019). Differences in the Conformational Energy Landscape of CDK1 and CDK2 Suggest a Mechanism for Achieving Selective CDK Inhibition. *Cell Chemical Biology* 26, 121-130.e5. doi: 10.1016/j.chembiol.2018.10.015

Xiao, X., Wang, W., Li, Y., Yang, D., Li, X., Shen, C., et al. (2018). HSP90AA1-mediated autophagy promotes drug resistance in osteosarcoma. *J Exp Clin Cancer Res* 37, 201. doi: 10.1186/s13046-018-0880-6

Yang, J., and Zhang, Y. (2015). I-TASSER server: new development for protein structure and function predictions. *Nucleic Acids Res* 43, W174-181. doi: 10.1093/nar/gkv342

Zhang, Y., Zhang, Y., Yun, H., Lai, R., and Su, M. (2014). Correlation of STAT1 with Apoptosis and Cell-Cycle Markers in Esophageal Squamous Cell Carcinoma. *PLoS ONE* 9, e113928. doi: 10.1371/journal.pone.0113928

Zhou, X., Wang, W., Li, Z., Chen, L., Wen, C., Ruan, Q., et al. (2022). Rosmarinic Acid Decreases the Malignancy of Pancreatic Cancer Through Inhibiting Gli1 Signaling. *Phytomedicine* 95, 153861. doi: 10.1016/j.phymed.2021.153861

Zhou, Y., He, X., Jiang, Y., Wang, Z., Yu, Y., Wu, W., et al. (2023). Repurposed benzydamine targeting CDK2 suppresses the growth of esophageal squamous cell carcinoma. *Front. Med.* 17, 290–303. doi: 10.1007/s11684-022-0956-8