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Research Article

Targeting Cell Cycle Checkpoints and Apoptotic Resistance in Lung Adenocarcinoma: An *In Silico* Approach to Natural Compound Therapeutics

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A B S T R A C T

Lung cancer remains a challenging because of its aggressive nature and limited treatment options. This study investigates the clinical significance of Chk1 and PARP1 overexpression in lung cancer and evaluated the potential natural compound as potential inhibitor. Bioinformatics analysis of TCGA data revealed significant upregulation of Chk1 and PARP1 in LUAD tissues compared to normal controls ($p<0.05$), high expression was found to be significantly correlated with poor overall survival. ADMET profiling was done to evaluate the pharmacological properties the compounds. Apigenin exhibited no Lipinski rule violations and was therefore selected for further studies. Molecular docking demonstrated strong binding of apigenin to both targets, with binding energies of -9.7 kcal/mol and -8.6 kcal/mol with Chk1 and PARP1 respectively, suggesting competitive inhibition at the ATP-binding and catalytic domains respectively. These computational findings align with existing experimental evidence of the ability of apigenin to induce G2/M arrest and sensitize cancer cells to DNA-damaging agents. This study proposes a novel therapeutic strategy combining apigenin with PARP inhibitors to obtain better patient outcomes in lung cancer cases by potentially overcoming chemoresistance. The findings of this study can further be validated by *in vitro*/ *in vivo* experiments to assess the efficacy of apigenin in modulating the Chk1/PARP1 pathways and improving treatment outcomes in lung cancer.

Keywords: Lung adenocarcinoma, Chk1, PARP1, apigenin, molecular docking, ADMET

Introduction

Lung cancer is a leading cause of cancer-related death worldwide. It is characterized by genetic and epigenetic alterations and dysregulation of essential cellular processes such as metabolism, cell cycle, and cell death mechanisms. Cancer cells undergo adaptations to ensure sustained proliferation by skipping cell cycle checkpoints and overcoming programmed cell death (apoptosis). Genetic alterations, such as mutations, anomalous expression of oncogenes and tumor suppressor genes, and dysregulation of signalling pathways are key phenomena involved in the transformation of normal cells into cancer cells. Finding the novel therapeutic targets and alternate therapies can be useful in developing better treatment approaches.[1,2,3]

Checkpoint kinase 1 (Chk1) and poly (ADP-ribose) polymerase 1 (PARP1) are well-studied molecular targets implicated in important physiological functions, and are often dysregulated in cancer. The DNA damage response (DDR) kinase ATR activates the serine/threonine kinase Chk1, which governs apoptosis by balancing pro- and anti-apoptotic proteins, such as Bcl-2 and imposes cell cycle arrest at the G2/M checkpoint to aid DNA repair.[4-6] In lung cancer, tumor cells utilize Chk1 to circumvent cell cycle checkpoints and evade apoptosis, a process fueled by replication stress and genomic instability owing to their rapid growth. Chk1 not only plays a role in the DNA damage response but also interacts with oncogenic pathways, such as PI3K/AKT and p53, boosting tumor survival and resistance to chemotherapy. It stabilizes p53 to trigger apoptosis and activates AKT to enhance cell survival. Chk1 inhibitors, such as LY2606368, are being tested in clinical trials alongside DNA-damaging agents, such as cisplatin to address resistance in advanced lung cancer.[7,8]

PARP1 is a nuclear enzyme that is vital for DNA repair through base excision repair (BER), emphasizing the link between DNA repair and cancer progression. It detects single-strand breaks (SSBs) and recruits repair proteins via poly(ADP-ribosyl)ation (PARylation). PARP1 not only aids DNA repair but also regulates apoptosis and cell cycle checkpoints. Moderate DNA damage activates PARP1 to promote survival, whereas excessive PARylation depletes NAD+/ATP, causing parthanatos, a caspase-independent cell death.[9,10] In lung cancer, overexpression of PARP1 paradoxically inhibits apoptosis by stabilizing anti-apoptotic proteins, such as Bcl-2, and inhibiting caspase-3, contributing to chemoresistance. PARP1 affects the G1/S transition through its interactions with p53 and E2F1, and its inhibition can cause G2/M arrest and mitotic catastrophe. PARP inhibitors, such as olaparib, exploit synthetic lethality in homologous recombination HR-deficient cancers by trapping PARP1 on DNA, creating lethal replication forks. In lung cancer, combining PARP inhibitors with Chk1 inhibitors increases DNA damage by disrupting both repair and checkpoint control, helping overcome resistance to platinum-based therapies. Natural compounds like curcumin and resveratrol enhance this strategy by downregulating PARP1, inducing apoptosis, and synergizing with chemotherapy, while offering lower toxicity than synthetic agents.[11-14]

In summary, Chk1 and PARP1 play crucial roles in the ability of lung cancer cells to evade apoptosis and disrupt normal cell cycle progression. Targeting both proteins simultaneously is a promising strategy to improve treatment effectiveness, especially in resistant cases. As clinical trials progress and natural compounds are being explored, incorporating Chk1 and PARP1 inhibitors into precision oncology could significantly enhance survival rates, providing new hope in the fight against this challenging disease.

Materials and Methods

Expression analysis using GEPIA2

The expression analysis of Chk1 and PARP1 was performed using the GEPIA2 web tool. The LAUD dataset was used for expression analysis, and the dataset was filtered to compare lung cancer tissues with GTEx normal tissues, applying a log2 fold change (logFC) threshold of 0.5 and a significant p-value cutoff of <0.05.

Survival analysis using KM Plotter

Survival analysis was performed using the Kaplan-Meier (KM) plotter tool. Chk1 and PARP1 expression data from patients with lung cancer were classified into high- and low-expression groups. Overall survival (OS) was investigated and statistical significance was evaluated using a log-rank test with a p-value threshold of <0.05.

ADMET analysis

The ADMET properties of the compounds were evaluated using SWISS ADME web tool (http://www.swissadme.ch). The SMILES notations of the compounds taken from PubChem were used to predict pharmacokinetic parameters, including gastrointestinal absorption, blood-brain barrier permeability, P-glycoprotein substrate status, and cytochrome P450 interactions. Drug-likeness was assessed using Lipinski's Rule of Five (≤1 violation). Bioavailability scores, synthetic accessibility, and toxicity endpoints (e.g., Ames mutagenicity and hepatotoxicity) were analyzed. These compounds with optimal pharmacokinetic profiles and minimal toxicity risks were selected for further investigation.

Molecular Docking

The crystal structures of CHK1 (PDB ID: 1ZYS) and PARP1 (PDB ID: 7ONT) were retrieved from the RCSB PDB. Protein preparation involved removing heteroatoms, adding polar hydrogens, and assigning Kollman charges using AutoDock Tools (v1.5.6). The binding pocket was defined by active-site residues (CHK1:Leu15, Val23, Leu84, Glu85, Leu137; PARP1: His862, Tyr907, Phe897, Ala898, Tyr907) with a 40×40×40 Å grid box centered on the catalytic domain. Apigenin (PubChem CID:5280443) was energy-minimized and converted into the PDBQT format. Docking was performed using AutoDock Vina (v1.2.0) with exhaustiveness of 8, generating 10 poses per ligand. The lowest-energy conformation (ΔG ≤ -8.5 kcal/mol) was analyzed for hydrogen bonds, hydrophobic contacts, and binding distances (<3.5 Å) using PyMOL (v2.5) and Discovery Studio (v2021)

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Result

Upregulation of Chk1 and PARP1 in LAUD cohort

Expression analysis box plots were generated using GEPIA2 to evaluate the roles of Chk1 and PARP1 in lung cancer. Expression analysis revealed a significant upregulation of Chk1 in lung tumor tissues compared to that in normal tissues. Stage plot analysis demonstrated a progressive increase in Chk1 expression with advancing tumor stage, suggesting its potential role in lung cancer progression and aggressiveness. Figure 1

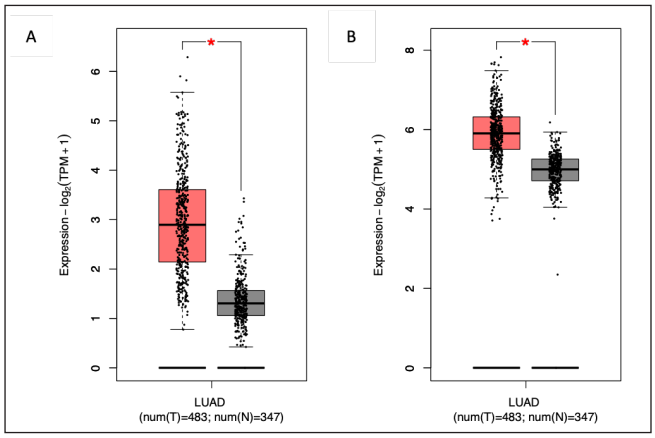

Figure 1.Box plots depicting the expression levels of (A) Chk1 and (B) PARP1 in Lung Adenocarcinoma (LUAD) tumor tissues (T) and normal tissues (N)

Chk1 and PARP1 are associated with poor survival in LAUD cohort

Kaplan-Meier survival analysis using the KM Plotter tool revealed a significant association between Chk1 expression

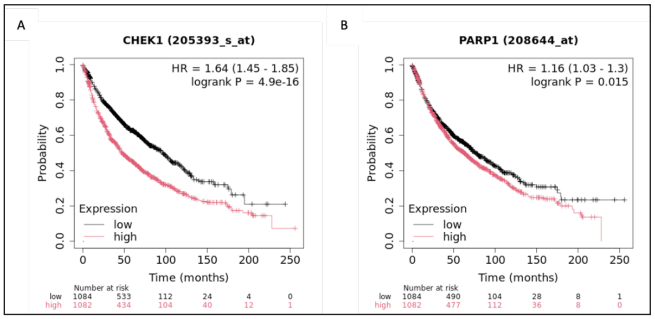

Figure 2.Overall Survival of LAUD cohort having high and low expression subgroups of (A) Chk1 (B) PARP1

and overall survival (OS) in patients with lung cancer. Patients with high Chk1 expression exhibited significantly poorer OS than those with low Chk1 expression ($p<0.001$)). Similarly, patients with higher PARP1 expression in lung cancer tissues had significantly poorer survival ($p=0.015$). These findings suggest that elevated Chk1 and PARP1 expression may serve as prognostic markers for reduced survival in patients with lung cancer. Figure 2

ADMET Analysis

SWISS ADME analysis showed distinct pharmacokinetic profiles for these compounds. Apigenin and quercetin had favorable drug-like properties, with 100% oral absorption, no Lipinski violations, and moderate water solubility. The ADMET analysis for top screened compounds are provided in Table1. These results indicate that apigenin as promising candidate for further studies, based on its pharmacokinetic properties.

Table 1.ADMET analysis and Pubchem ID of selected natural compounds

S.no.	Compound Name	Pubchem ID	Molecular weight (g/mol)	log p	H bond donor	H bond acceptor	Lipinski violations
1.	Apigenin	5280443	270.24	2.11	3	5	0
2.	Quercetin	5280343	302.24	1.23	5	7	0
3.	Isoquercetin	5280804	464.38	0.48	8	12	2
4.	Myricetin	5281672	318.24	0.79	8	6	1
5.	Levomenol	442343	222.37	3.79	1	1	0
6.	Columbianin	13989896	570.54	1.02	7	14	3
7.	Cirsilineol	162464	344.32	2.53	2	7	0
8.	Cannabiscitrin	5486615	480.38	0.86	9	13	2
9.	Aromadendrine	122850	288.25	4.34	4	6	0
10.	Amentoflavone	5281600	538.46	3.62	6	10	2

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Apigenin shows high binding affinity with Chk1 and PARP1

The docking results showed that both CHK1 and PARP10 exhibited good binding affinity with Apigenin, with binding energies of -9.7 kcal/mol and -8.6 kcal/mol, respectively, indicating strong and stable interactions. This high binding affinity suggests that apigenin effectively interacts with key residues in the active sites of both proteins via hydrogen bonding, hydrophobic interactions, and π–π stacking. Since CHK1 and PARP1 are known to play crucial roles in cancer cell survival and DNA repair, the strong binding of apigenin to these targets highlights its potential as a therapeutic agent for lung cancer treatment. Figure 3

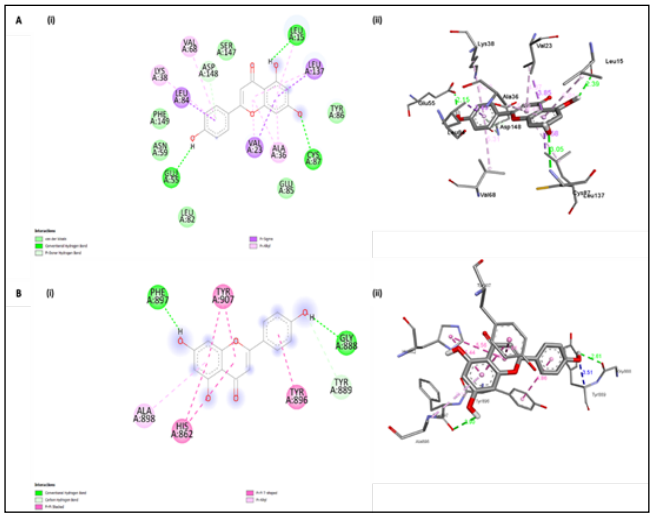

Figure 3.Molecular docking studies showing the binding affinity of apigenin with: (A-i) illustrates the two-dimensional diagram of all the interacting amino acids of CHK1 with apigenin. (A-ii) details the amino acids interacting with CHK1 and the bond lengths with apigenin. (B-i) Two-dimensional diagram of all the interacting amino acids of PARP1 with apigenin. (B-ii) specifies the amino acids interacting with PARP1 and the bond lengths with apigenin

Discussion

Lung cancer is one of the most prevalent and aggressive types of cancer with poor patient outcomes and limited treatment options. Therefore, it is crucial to explore novel therapeutic targets and develop better therapeutic strategies for the treatment of this disease. In this study, we investigated the expression of CHK1 and PARP1 in LUAD and their significance in patient survival. Furthermore, natural compounds were screened for their potential as therapeutic agents that target Chk1 and PARP1. We found significant upregulation of CHK1 and PARP1 in LUAD tissues compared to normal tissues, which is consistent with previous studies suggesting their elevated expression in various cancers, including lung, gastric, and breast cancers. CHK1is known to a crucial role in DNA damage response (DDR) pathway and is important for cell cycle checkpoint control and genomic integrity. Its high expression has been reported to be positively associated with tumor progression, resistance to chemotherapy, and poor prognosis. Similarly, PARP1, known for its role in DNA repair through the base excision repair (BER) pathway, is overexpressed in tumors with defective DDR, thereby promoting cancer cell survival.[15-18]

We performed Kaplan-Meier survival analysis to correlate the high expression levels of CHK1 and PARP1 with disease prognosis and found that both genes were significantly associated with poor overall survival (OS) in LUAD patients. Our findings are consistent with those of previous studies, suggesting that CHK1 overexpression is associated with aggressiveness and poor prognosis in lung cancer. The significant association between elevated PARP1 expression and poor OS also corroborates with previous studies showing that PARP inhibitors such as Olaparib, Niraparib exhibit promising outcomes in lung cancer treatment.[15,19,20]

To explore potential therapeutic inhibitors targeting CHK1 and PARP1, several natural compounds were screened. We performed an ADMET analysis to evaluate the pharmacokinetic properties of the selected compounds. Apigenin and quercetin were found to have optimal drug-like properties, with high oral absorption, moderate water solubility, and favorable metabolic stability. Apigenin is a natural flavonoid known for its antiproliferative, pro-apoptotic, and chemosensitizing properties in various cancers.[21,22] Our findings are consistent with those of previous studies that demonstrated the properties of apigenin in selectively targeting cancer cells with low toxicity to normal cells, making it a suitable molecule for further drug development.

Furthermore, we conducted molecular docking studies to investigate the interaction of apigenin with the selected targets. Our findings revealed high binding affinities of apigenin with CHK1 (-9.7 kcal/mol) and PARP1 (-8.6 kcal/mol), indicating their stable interactions. Previous studies have suggested that apigenin promotes cell cycle arrest, apoptosis, and oxidative stress-mediated DNA damage in lung cancer cells.[22,23] Moreover, apigenin may enhance the efficacy of chemotherapeutic agents by inhibiting DDR pathways, suggesting its potential role as a CHK1 and PARP1 inhibitor.

Overall, our study highlights the therapeutic potential of apigenin as an inhibitor of CHK1 and PARP1 and can provide a novel therapeutic approach for lung cancer

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treatment. It is well documented that CHK1 and PARP1 inhibitors exhibit efficient role in cancer therapies, and combining apigenin with standard chemotherapeutic agents can increase treatment efficacy. Further *in vitro* and *in vivo* validation is essential to confirm these interactions and to evaluate the functional impact of apigenin on LUAD cell proliferation, apoptosis, and DNA repair pathways.

In conclusion, our findings reveal elevated expression of CHK1 and PARP1 in LUAD, which are associated with poor prognosis. The strong binding affinity of apigenin to target proteins suggests that it may serve as a promising lead compound for targeted therapy. Future studies integrating preclinical models and clinical trials are necessary to establish its efficacy as a potential anticancer agent for LUAD treatment.

Conflicts of Interests: None

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

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