Original Research Paper

Volume - 15 | Issue - 11 | November - 2025 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Bio-medical

INTEGRATED BIOINFORMATICS ANALYSIS AND DRUG SCREENING FOR NOVEL TARGETS IN CERVICAL CARCINOMA: FOCUSING ON DIFFERENTIALLY EXPRESSED HUB GENES

Ms. P. Bharani	Department Of Biochemistry & Nutrition, Bhavan's Vivekananda College Of Science, Humanities & Commerce, Sainikpuri, Secunderabad, Telangana - 500 094, India.
Ms. P. Sai Teja Vaishnavi	Department Of Biochemistry & Nutrition, Bhavan's Vivekananda College Of Science, Humanities & Commerce, Sainikpuri, Secunderabad, Telangana - 500 094, India
Ms. B. Maithri	Department Of Biochemistry & Nutrition, Bhavan's Vivekananda College Of Science, Humanities & Commerce, Sainikpuri, Secunderabad, Telangana - 500 094, India.
Dr. S. Padma	Head, Department of Biochemistry & Nutrition, Bhavan's Vivekananda College of Science, Humanities and Commerce, Sainikpuri, Secunderabad, Telangana- 500095, India

ABSTRACT The present study aims to identify differentially expressed genes in cervical cancer from publicly available datasets and identify probable drug targets using *insilico* bioinformatics tools. Three datasets from the NCBI GEO database were retrieved and analyzed to determine differentially expressed genes with their protein-protein interactions mapped using STRING and visualized in Cytoscape. This was followed by a comparison with cervical cancer patient data from cBioPortal. The selected proteins were evaluated for potentially harmful mutations using SIFT and PolyPhen tools. Druggability of these target proteins was assessed using DOGSITE SCORER and SWISSTARGET PREDICTION, with subsequent molecular docking performed via AutoDock tools. Analysis of GEO datasets using GEO2R, STRING and Cytoscape identified 10 significant upregulated genes including CCND1. Data from cBioportal indicated amplification of CCND1 in 3% cases and mutational analysis using SIFT and Polyphen revealed damaging mutations in CCND1 and was thus selected for drug targeting. The present study concludes that CCND1 is a putative drug target and invitro and invivo studies can confirm the effect of ellagic acid and quercetin on CCND1 activity. The study furthers gains relevance as the dysregulation of CCND1 in cancers other than cervical cancer is well reported. Further, practical utility of the present study implies the use of nutraceuticals as food supplements or adjuvant treatments to modulate CCND1 expression.

KEYWORDS : Differentially expressed genes, CCND1, mutations, drug targeting

INTRODUCTION

Cervical cancer, a significant global health issue, is the fourth leading cause of cancer-related deaths in women, with a mortality rate of 9.1 in India, where it ranks as the third most common cancer (GLOBOCAN 2020). It disproportionately affects economically disadvantaged areas due to inadequate sanitation and limited healthcare access. (Ramamoorthy *et al.*, 2024). Persistent infection with high-risk human papillomavirus (HPV) is the primary cause, but additional factors are required for cancer development. These include long-term use of hormonal contraceptives, multiple pregnancies, early sexual debut, multiple partners, smoking, and HIV co-infection, which promote HPV persistence or weaken immune responses. Other potential risk factors, such as co-infections with Chlamydia trachomatis or Herpes simplex virus type-2, immune suppression, poverty, poor hygiene, and diets lacking antioxidants, may worsen inflammation or impair immunity. (Wu X *et al.*, 2019). The disease mainly presents as squamous cell carcinoma (about 80% of cases) or adenocarcinoma, originating in the squamous epithelial cells of the cervical transformation zone (Gadkari R *et al.*, 2022). Screening relies on methods like the Pap smear, high-risk HPV testing, and colposcopy. Cervical cancer develops progressively from low-grade cervical intraepithelial neoplasia (CIN1) to high-grade CIN (CIN2/3) and eventually carcinoma, driven by complex genetic and epigenetic alterations, including oncogene activation and tumor suppressor gene silencing. Bioinformatics approaches have emerged as powerful tools to study cervical cancer progression. High-throughput technologies, such as microarrays identify differentially expressed genes (DEGs) linked to tumorogenesis, revealing potential biomarkers and therapeutic targets (Balasubramaniam SD *et al.*, 2019). Analysis of gene expression, protein interaction networks, and key hub genes provides insights into the molecular pathways driving cervical cancer. These findings are crucial for developing targeted treatments and the present study thus aims to identify the key genes which could be targeted by neutraceuticals that are potent antioxidants and can improve immunity to infections.

MATERIALS AND METHODS

Cervical squamous cell carcinoma datasets with accession numbers GSE27678 (Caffareel *et al*, 2013), GSE9750 (Scotto *et al*, 2008), GSE122697 (Roychowdhury *et al*, 2020) were selected from the GEO database of NCBI. Each dataset was analysed for differentially expressed genes (DEGs) using GEO2R. The genes with log Fc>1 from the three different sets were combined into a single set and the common genes from all the different sets were used for further analysis. Functional enrichment analysis of the differentially expressed genes was performed using gProfiler. (Wu K *et al.*, 2018). The curated up regulated & down regulated genes were analysed using the STRING database. Interactions with a confidence score > 0.4 (medium or advanced) were considered significant. Protein – protein interaction data obtained from the STRING database were imported into Cytoscape to construct an interaction network and analyse network topology. Mutational analysis of proteins was performed using PolyPhen- 2 (Polymorphism Phenotyping v2) and SIFT (Sorting Intolerant From Tolerant) tools. cBioPortal data from patients with squamous cell carcinoma of cervix were checked to assess the frequency and distribution of inheritable alterations such as mutations, amplifications, and deletions across genes identified by DEG analysis. Copy number variations (CNV) profiles of the hub genes identified by cytoscape analysis and overall survival status were visualized using OncoPrint and heat charts. The protein CCND1 identified to have deleterious mutations was then studied for Structure and Ligand based druggability using DoGSiteScorer and Swiss Target Prediction tools. Molecular docking studies were performed to investigate the binding affinity and interaction profiles of ellagic acid and quercetin with the Cyclin D1 (CCND1) protein using AutoDock Vina. The 3D structure of CCND1 was retrieved from the Protein Data Bank (PDB), and ligand structures were obtained from PubChem and prepared using Open Babel. Docking was carried out within a defined grid box covering the active point predicted from DoGSiteScorer. The molecular docking results of ellagic acid and quercetrin with Cyclin D1 (CCND1) were visualized using BIOVIA Discovery Studio Visualizer. The docked complexes attained from AutoDock Vina were imported into the software to analyses ligand – protein interactions in detail. Crucial interactions, including hydrogen bonds, hydrophobic connections, $\pi - \pi$ stacking, and van der Waals forces, were linked and visually represented. 2D interaction plates were generated to easily illustrate the binding mode of each ligand within the CCND1 active site. 3D surface views were used to confirm the ligand fitting within the binding pocket, further supporting the docking results.

RESULTS & DISCUSSION

Gene expression analysis for different upregulated and downregulated genes in cervical cancer was carried out on three publicly available datasets, GSE27678, GSE9750 and GSE122697 using GEO2R. The top 10% differentially expressed genes (DEGs) from each data set were then curated to identify the common genes across all three

different data sets. A total of 470 upregulated and 438 downregulated genes common across the 3 datasets were identified. Functional enrichment analysis of the upregulated genes demonstrated that the gene enrichment was more in the transcriptional regulator complex, cornified envelope, euchromatin, and nucleoplasmic reticulum and associated with molecular functions like regulation of miRNA transcription, tissue development, positive regulation of fibroblast proliferation, and response to light and endogenous stimuli. transcriptional coregulator binding, DNA-binding transcription activator activity, R-SMAD binding, and core promoter sequence-specific DNA binding (Fig1).

Fig 1 : Functional Enrichment Analysis of upregulated genes

Functional enrichment analysis of the downregulated genes revealed enrichment in chromosomal region, nuclear chromosome, catalytic complex, and nucleoplasm suggesting reduced activity in nuclear and chromosomal functions. Further enriched downregulated genes included those associated with DNA replication, chromosome organization, DNA damage response, and nuclear division, indicating a suppression of cell cycle and genomic maintenance activities (Fig2)

Fig 2 : Functional Enrichment Analysis of downregulated genes

The protein-protein interaction (PPI) network of the upregulated DEGs displayed a relatively moderate complexity indicating a broad but less densely connected network of protein interactions that may be involved in regulatory or developmental processes. Conversely, the downregulated DEGs formed a highly interconnected network, reflecting a more compact and densely interactive protein group. This dense network suggests that these proteins might participate in tightly coordinated biological pathways, such as those involved in cell cycle regulation, DNA repair, or replication. The top 10 hub genes of cervical squamous cell carcinoma found to be upregulated were ESR1, JUN, IGF1, MYC, FOS, CCND1, KRT14, IVL, DSG1 and CALM15 and 10 significant down regulated were CDK1, CCNA2, BRCA2, RAD51, CCNB1, EXO1, CHEK1, CDC6, CDC45 and TOP2A. Hub genes were linked based on high degree values, suggesting their potential regulatory significance. The hub genes identified were then analysed for any genomic alterations, including mutations, and copy number variations (CNVs) in the squamous cell cervical cancer samples in cBioportal repository. The OncoPrint analysis of the analyzed genes, showed notable alterations in amplification and deletion frequencies. Amongst the genes, CCND1 exhibited amplification in approximately 3% of cases, indicating its potential role in promoting tumor proliferation and this gene also showed instances of deep deletion, suggesting heterogeneity in its genomic regulation across different patients. This amplification increases the gene dosage, resulting in the overexpression of Cyclin D1 protein. High expression of CCND1 is correlated with poor prognosis of patients with cervical cancer. (Wang *et al*,2023). Proposed role of CCND1 in the progression of cervical cancer is indicated in Fig3. The pathway suggests that CCND1 could cause hyperphosphorylation of Retinoblastoma 1 and thus lead to its inactivation. (Wang *et al*,2023).

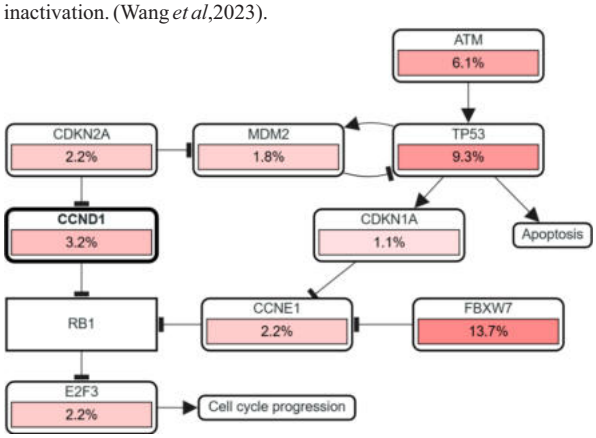

Fig 3 : The pathway of CCND1

Mutational analysis of differentially expressed upregulated and downregulated genes revealed that CCND1 exhibited the highest SIFT score of 1, suggesting it is likely deleterious and may play a critical role in cervical cancer progression. Mutational analysis identified a potentially damaging mutation, P287T, in the CCND1 gene. Prior research has extensively documented CCND1 mutations, particularly the G870A single nucleotide polymorphism (SNP), due to its function as a key cell cycle regulator and oncogene. However, the G870A polymorphism in CCND1 appears to have little to no association with cervical cancer risk (Xue *et al*, 2015; Hu *et al.*, 2014). The 3D structure of CCND1, recaptured from the Protein Data Bank (PDB), was uploaded to the DoGSiteScorer platform to identify potential binding pockets. The tool analyses geometrical and physicochemical properties of detected pockets, such as volume, depth, hydrophobicity, and enclosure, and assigns a druggability score ranging from 0 (non-druggable) to 1 (highly druggable). Pockets with higher scores were considered promising sites for small molecule binding, providing valuable insights for future structure- based drug design targeting CCND1. The DOGSITE analysis reveals that the CCND1-2W99 protein structure has a high druggability score (0.8), indicating it's a good target for drug discovery. Its binding pocket, with a volume of 1126.33 Å and a mix of hydrogen bonding and hydrophobic features, offers promising interaction sites for potential drug. (Fig 4).

Fig 4: Druggability pocket of CCND1

Swiss Target Prediction tool was used to identify natural compounds

that could potentially target CCND1 based on chemical structure similarity and known drug interactions (Ning *et al.*, 2025). The phytochemicals, ellagic acid and quercetin, earlier reported to inhibit crucial pathways in cervical cancer cell lines (Xia *et al*, 2020, Pani *et al*, 2021), showed the highest probability of binding to CCND1. Molecular docking between CCND1 and Ellagic acid & Quercetin drug molecules were carried out using AutoDockVina and visualized. Ellagic acid has slightly highest interaction and lowest binding affinity i.e. -7.9 (kcal/mol) than Quercetin i.e. -7.4 (kcal/mol). Results showed strong interactions of CCND1 with ellagic acid and Quercetin, indicating their potential as therapeutic agent. (Fig5&6).

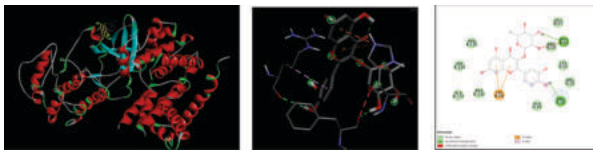

Fig 5 : The docking of Ellagic with the CCND1 (2D, 3D Structure & types of bonds).

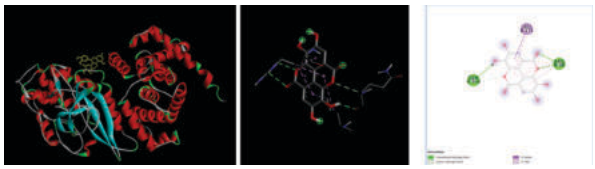

Fig 6: The docking of Quercetin with the CCND1 (2D, 3D Structure & types of bonds).

The main observations in the present study reports that CCND1 emerges as a compelling hub gene in cervical cancer, showing amplification in about 3% of the squamous cell carcinoma samples. This amplification, together with deleterious mutations identified by SIFT/PolyPhen, positions CCND1 not just as a marker of aberration but as a plausible driver of malignant behaviour. The findings align with prior literature which reports that CCND1 amplification or overexpression is common in many cancers and often correlates with poorer prognosis and more aggressive features. (Chen Yu *et al.*, 2020). On the therapeutic side, the interactions of CCND1 with natural compounds like ellagic acid and quercetin, as evident from molecular docking results, are promising. These compounds have been shown to inhibit proliferation, induce apoptosis, and downregulate CCND1 expression in cervical cancer and other cancer types. (Wang J *et al.*, 2023). These studies suggest that targeting CCND1 via such natural compounds might not only suppress tumor proliferation but also help overcome resistance or reduce side effects of conventional therapies. However, these therapeutic potential needs validation in vivo and in clinical settings. Additionally, dosage, bioavailability, and specificity are important caveats to be addressed in future investigations. This study provides a comprehensive bioinformatics analysis of cervical cancer microarray data, identifying key differentially expressed genes and highlighting CCND1 as a potential hub gene with notable amplification and damaging mutations. Structural and ligand-based assessments, along with molecular docking, revealed strong druggability of CCND1 and potential interactions with natural compounds such as ellagic acid and quercetrin, suggesting its promise as a therapeutic target.

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