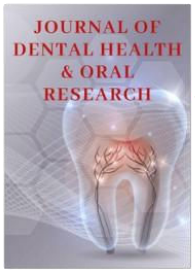

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Research Article

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Bio-Informatic Analysis of Protein Kinase-C in Oral Squamous Cell Carcinoma Through Network Topology: An In-Silico Study

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Abstract

Background: Protein Kinase C (PKC) is a family of serine/threonine kinases that regulate diverse signalling pathways involved in cancer development, including Oral Squamous Cell Carcinoma (OSCC). This study aims to investigate the molecular interaction network of PKC in OSCC, identify potential drug targets through pan-drug analysis and evaluate therapeutic candidates using molecular docking.

Materials and Methods: Gene and protein sequence data related to PKC and OSCC were obtained from publicly available databases. Overlapping targets were identified and used to construct a Protein-Protein Interaction (PPI) network using STRING and Cytoscape. Functional enrichment and network topology analyses identified key hub genes. Drug-gene interactions were analysed using PanDrugs and the top-ranked drug, CETUXIMAB, was subjected to molecular docking against PKC Alpha (PRKCA) using AutoDock Vina. Ligand-protein interactions were evaluated for binding affinity and hydrogen bonding.

Results: Out of 4945 OSCC-associated and 101 PKC-related genes, 64 common targets were identified. EGFR, ERBB2 and PIK3CA were key hubs in the PPI network. CETUXIMAB showed the highest DScore (1), targeting multiple oncogenic proteins including PRKCA. Docking analysis revealed a binding energy of -5.9 kcal/mol with three hydrogen bonds at Val A:420, Val A:353 and Asp A:481, indicating strong interaction.

Conclusion: This in-silico study highlights the role of PKC in OSCC and identifies CETUXIMAB as a promising therapeutic candidate for repurposing. The findings support further experimental and clinical validation to explore PKC-targeted interventions in oral cancer.

Keywords: Protein Kinase C; Oral Squamous Cell Carcinoma; Gene Ontology; Molecular Docking; Pan-Drug Analysis; Protein-Protein Interaction; Cetuximab

Introduction

Oral Squamous Cell Carcinoma (OSCC) is among the most prevalent and aggressive malignancies affecting the head and neck region, accounting for a significant proportion of cancer-related morbidity and mortality worldwide [1]. Despite advances in

diagnostic and therapeutic strategies, the prognosis of OSCC remains poor due to late-stage detection, metastasis and resistance to conventional therapies. Understanding the molecular mechanisms underlying OSCC progression is essential for the identification of novel diagnostic markers and therapeutic targets [2].

Protein Kinase C (PKC) is a family of serine/threonine kinases that play a central role in regulating diverse cellular processes, including cell proliferation, differentiation, apoptosis and immune responses. PKC isozymes are activated by various stimuli such as Diacylglycerol (DAG) and calcium ions and are implicated in several disease pathways, particularly cancer, neurodegenerative disorders and cardiovascular diseases [3]. Understanding the molecular interactions and potential therapeutic targeting of PKC is essential for the development of effective pharmacological interventions. Protein-Protein Interactions (PPIs) involving PKC are crucial for its localization, activation and function within the cell. PKC interacts with a wide range of proteins, including scaffolding proteins (e.g., RACKs - Receptors for Activated C-Kinase), cytoskeletal elements, transcription factors and other kinases. These interactions often dictate the specificity and outcome of PKC signalling pathways [4]. Bioinformatics tools and interaction databases such as STRING, BioGRID and IntAct can be used to map the interactome of PKC. Analysis of these interactions helps in identifying potential co-regulators and signalling networks in which PKC is involved. Molecular docking is a computational technique that predicts the preferred orientation of one molecule (typically a small drug-like compound) to a second (such as a protein target) to form a stable complex [5]. In the context of PKC, molecular docking allows the exploration of potential inhibitors or modulators that can selectively bind to the catalytic or regulatory domains of various PKC isoforms. Docking studies often use crystallographic structures of PKC retrieved from the Protein Data Bank (PDB). Ligands are virtually screened using tools such as AutoDock, Glide or MOE to evaluate their binding affinities and interaction profiles [6]. These in-silico experiments provide insight into the binding pockets, hydrogen bond formation, hydrophobic interactions and potential inhibitory activity of candidate compounds [7].

Pan-drug analysis refers to evaluating the interaction and susceptibility of a single protein target across a broad spectrum of pharmacological agents. For PKC, this type of analysis is particularly valuable for identifying multi-targeted kinase inhibitors or designing isoform-specific drugs to avoid undesirable off-target effects. Using platforms like DGIdb (Drug Gene Interaction Database) or PharmMapper, researchers can identify known and predicted drugs that may modulate PKC activity [8,9]. Pan-drug analysis assists in categorizing compounds into activators, inhibitors or modulators and assesses their pharmacodynamic and pharmacokinetic profiles. This process is instrumental in drug repurposing efforts, especially in complex diseases like cancer, where PKC may contribute to drug resistance mechanisms [10,11].

The Protein-Protein Interactions (PPIs) involving PKC can provide valuable insights into its functional associations and influence within the molecular network of OSCC [12]. Additionally, molecular docking approaches allow for the virtual screening of compounds that may modulate PKC activity, paving the way for the development of targeted therapies [13]. A pan-drug analysis further enables the assessment of PKC's responsiveness to a wide range of pharmacological agents, facilitating drug repurposing and personalized treatment strategies. This in-silico study aims to explore the role of PKC in OSCC through an integrated approach involving network topology-based analysis of PPIs, molecular docking and pan-drug profiling. By leveraging bioinformatics and computational tools, this study seeks to uncover potential drug candidates and deepen our understanding of PKC's molecular landscape in the pathogenesis of OSCC [14].

Methodology

This In-silico study was conducted to explore the interaction network of Protein Kinase C (PKC) in Oral Squamous Cell Carcinoma (OSCC) and to identify potential therapeutic candidates through molecular docking and drug-target interaction analysis.

1. Gene and Protein Data Retrieval

Gene and protein sequences for PKC isoforms (α, β, δ, ε) were retrieved from UniProt and NCBI databases. Additional OSCC-related genes were identified via GeneCards and The Human Protein Atlas. This dataset served as the basis for interaction and functional analyses.

2. Protein-Protein Interaction Network Construction

PPI data for Homo sapiens were obtained from the STRING database using a confidence score cutoff of 0.7. The resulting network was visualized and analysed in Cytoscape (v3.9.1) using NetworkAnalyzer and CytoHubba to identify key hub proteins associated with OSCC and PKC.

3. Functional and Pathway Enrichment

Gene Ontology (GO) and KEGG pathway analyses were conducted using DAVID and KEGG tools to evaluate biological processes, molecular functions and pathways relevant to carcinogenesis, including signal transduction, apoptosis and EMT.

4. Pan-Drug and Therapeutic Target Analysis

Drug-target interactions were explored using DGIdb and PharmGKB. Drug candidates were filtered for relevance to cancer and their pharmacological properties were evaluated using SwissADME. Lipinski's criteria and ADME profiles were considered to identify promising small molecules.

5. Molecular Docking Workflow

Generated protein models were utilized for the process of docking. Protein three-dimensional structure was analysed for error using pymol software. Similarly, the ions molecules and standard inhibitors if present were also removed using pymol visualization tool. The Chem3D ultra 11.0 software was used to construct the three-dimensional structures of the ligands to be studied. Kollmann charges, polar hydrogen bonds were added to the protein, simultaneously all bound water and ligands were removed. Modelling of the drug compound i.e ligand molecule was carried out using java script based Marvin Sketch software. Protein Preparation: 3D structures of PKC isoforms and selected hub proteins were sourced from RCSB PDB or generated using SWISS-MODEL. Structures were cleaned and pre-processed using latest version of Auto Dock Vina v.1.2.0. software was used for the automated docking study. The auto grid, component of auto dock, was used to compute the grid maps with the interaction energies depending upon the macromolecule target of the docking study. The grid centre was placed on the active target site region of the enzyme. Then binding free energy of the inhibitors was evaluated using automated docking studies. The best conformations search was done by adopting Genetic Algorithm with Local Search (GA-LS), method. The docking parameters were set default values with 100 independent docking runs using the software ADT (Auto-Dock Tool Kit). Root Mean Square (RMS) tolerance of 2.0 Å was performed using structures generated after completion of docking via cluster analysis. Molecular graphics and visualization were performed with the Discovery studio visualizer tool. 100 evaluations were carried for each ligand with all protein targets and the posed.

6. Integration and Candidate Prioritization

Docking results were integrated with network and pharmacological data to identify the most promising compounds.

Results

A total of 4945 genes associated with Oral Squamous Cell Carcinoma (OSCC) and 101 genes interacting with Protein Kinase C (PKC) were identified, with 64 overlapping genes (Fig. 1, Table 1 ,2). These common targets were analysed using PPI network topology, gene ontology and pan-drug screening to evaluate their relevance in OSCC progression and therapeutic potential.

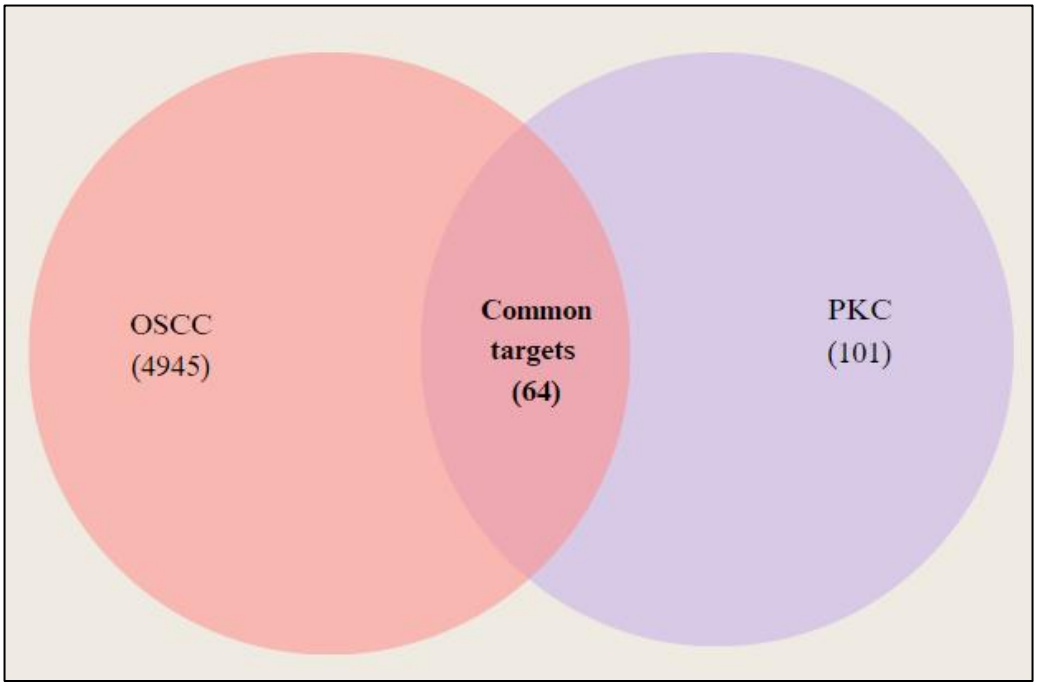

Figure 1: Schematic representation of Common genes among Gene cards and PKC string analysis.

S.No	OSCC No of Targets	Gene Targets of PKC	Common Targets with Protein Kinase C
1	4945	101	64

Table 1: Common targets between PKC and SOCC.

S.No	Target Name	Uniprot ID	Target Genes
1	Actin Beta	P60709	ACTB
2	B Cell Linker	Q8WV28	BLNK
3	B-Raf Proto-Oncogene, Serine/Threonine Kinase	P15056	BRAF
4	Caspase 3	P42574	CASP3
5	Cbl Proto-Oncogene	P22681	CBL
6	Cadherin 1	P12830	CDH1
7	CF Trans membrane Conductance Regulator	P13569	CFTR
8	Cytochrome B-245 Alpha Chain	P13498	CYBA
9	Decorin	P07585	DCN
10	Discs Large MAGUK Scaffold Protein 4	P78352	DLG4
11	EGF Containing Fibulin Extracellular Matrix Protein 2	O95967	EGF
12	Epidermal Growth Factor Receptor	P00533	EGFR
13	Erb-B2 Receptor Tyrosine Kinase 2	P04626	ERBB2
14	Erb-B2 Receptor Tyrosine Kinase 3	P21860	ERBB3
15	Epiregulin	O14944	EREG
16	Ezrin	P15311	EZR
17	Fos Proto-Oncogene, AP-1 Transcription Factor Subunit	P01100	FOS
18	FosB Proto-Oncogene, AP-1 Transcription Factor Subunit	P53539	FOSB
19	GRB2 Associated Binding Protein 1	Q13480	GAB1
20	G Protein Subunit Alpha Q	P50148	GNAQ
21	Heparin Binding EGF Like Growth Factor	Q99075	HBEGF
22	HRas Proto-Oncogene, GTPase	P01112	HRAS
23	Integrin Subunit Alpha 5	P08648	ITGA5
24	Integrin Subunit Alpha V	P06756	ITGAV
25	Integrin Subunit Beta 1	P05556	ITGB1
26	Inositol 1,4,5-Trisphosphate Receptor Type 1	Q14643	ITPR1
27	JunD Proto-Oncogene, AP-1 Transcription Factor Subunit	P17535	JUND
28	KRAS Proto-Oncogene, GTPase	P01116	KRAS
29	Mitogen-Activated Protein Kinase Kinase 2	P36507	MAP2K2
30	Mitogen-Activated Protein Kinase 10	P53779	MAPK1
31	Mitogen-Activated Protein Kinase 3	P27361	MAPK3
32	Mechanistic Target of Rapamycin Kinase	P42345	MTOR
33	NRAS Proto-Oncogene, GTPase	P01111	NRAS
34	Neuregulin 1	Q02297	NRG1
35	Platelet Derived Growth Factor Receptor Beta	P09619	PDGFRB
36	3-Phosphoinositide Dependent Protein Kinase 1	O15530	PDPK1
37	Profilin 1	P07737	PFN1
38	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	P42336	PIK3CA
39	Phospholipase C Beta 1	Q9NQ66	PLCB1
40	Phospholipase C Beta 4	Q15147	PLCB4
41	Phospholipase C Epsilon 1	Q9P212	PLCE1
42	Phospholipase C Gamma 1	P19174	PLCG1
43	Phospholipase D1	Q13393	PLD1

44	Phospholipase D2	O14939	PLD2
45	Protein Phosphatase 2 Catalytic Subunit Alpha	P67775	PPP2CA
46	Protein Phosphatase 2 Regulatory Subunit B'Alpha	Q15172	PPP2R5A
47	Protein Kinase C Alpha	P17252	PRKCA
48	Protein Kinase C Beta	P05771	PRKCB
49	Protein Kinase C Delta	Q05655	PRKCD
50	Protein Kinase C Gamma	P05129	PRKCG
51	Protein Tyrosine Kinase 2	Q05397	PTK2
52	Paxillin	P49023	PXN
53	Receptor For Activated C Kinase 1	P63244	RACK1
54	RAS P21 Protein Activator 1	P20936	RASA1
55	Ras Homolog, MTORC1 Binding	Q15382	RHEB
56	Ribosomal Protein L29	P47914	RPL29
57	Ribosomal Protein L35	P42766	RPL35
58	Ribosomal Protein S27a	P62979	RPS27A
59	Ribosomal Protein S3	P23396	RPS3
60	Ribosomal Protein S6 Kinase B1	P23443	RPS6KB1
61	Syndecan 4	P31431	SDC4
62	Sphingosine Kinase 1	Q9NYA1	SPHK1
63	Transient Receptor Potential Cation Channel Subfamily V Member 4	Q9HBA0	TRPV4
64	Vascular Cell Adhesion Molecule 1	P19320	VCAM1

Table 2: Common genes among Gene cards and PKC string analysis.

Data Mining: Gene and Protein Targets of PKC

A total of 101 genes are identified as Gene/protein said to have ineteraction with protein kinase C. Imporatnt gene and protein targets of PKC are listed below encompass a diverse array of molecules involved in various cellular processes.

Some notable targets include:

- Signal Transduction Molecules: GAB1, RACK1, RHEB, RASA1, EGFR, ERBB2, ERBB3, GNAQ, HRAS, KRAS, NRAS and RAS family members. These proteins are involved in transmitting extracellular signals to intracellular pathways, regulating cell growth and differentiation
- Transcription Factors: FOS, FOSB, JUND and AP-1. These factors regulate gene expression and are implicated in processes like cell proliferation, differentiation and response to stress
- Cell Adhesion and Migration Molecules: ITGA1, ITGA5, ITGAV, ITGB1, SDC4, VCAM1 and EZR. These molecules play roles in cell adhesion, migration and cytoskeletal organization
- Receptors and Growth Factors: EGFR, ERBB2, ERBB3, EGF, NRG1, HBEGF and PDGFRB. These receptors are involved in growth factor signaling and regulation of cell growth, survival and differentiation
- Ion Channels: CACNA1C, CACNA1F, CACNA1S, TRPC3 and TRPV4. These channels play a role in cellular excitability, calcium signaling and regulation of various physiological processes
- Cell Cycle and Apoptosis Regulators: BRAF, CASP3, DEPTOR, MAP2K2, MAPK1, MAPK3, MAPKAP1, MTOR, PDPK1 and RPS6KB1. These proteins are involved in cell cycle progression, apoptosis regulation and intracellular signaling pathways

Disease Specific Target Prediction

Squamous Oral Cell Carcinoma (SOCC) is a complex disease with multiple genetic alterations involved in its development and progression. Gene card databases was used for predicting disease specific targets. A total of 4945 genes were identified as gene and protein targets for Squamous Oral Cell Carcinoma (SOCC)

- TP53 (Tumor Protein 53): TP53 is a tumor suppressor gene that regulates cell cycle arrest, DNA repair and apoptosis. Mutations in TP53 are frequently observed in OSCC
- CDKN2A (Cyclin-Dependent Kinase Inhibitor 2A): CDKN2A encodes proteins p16INK4a and p14ARF, which are involved in cell cycle regulation. Alterations in CDKN2A are associated with SOCC development

- EGFR (Epidermal Growth Factor Receptor): EGFR is a receptor tyrosine kinase involved in cell proliferation and survival. Overexpression or activating mutations in EGFR are commonly observed in OSCC
- HRAS (Harvey Rat Sarcoma Viral Oncogene Homolog): HRAS is a member of the RAS gene family that regulates cell growth and differentiation. Activating mutations in HRAS are found in a subset of OSCC cases
- PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha): PIK3CA encodes the catalytic subunit of PI3K, a signaling molecule involved in cell growth and survival. Mutations in PIK3CA are implicated in OSCC progression
- NOTCH1 (Notch Receptor 1): NOTCH1 is a transmembrane receptor involved in cell fate determination. Mutations in NOTCH1 have been reported in OSCC, affecting cell differentiation and proliferation
- CCND1 (Cyclin D1): CCND1 is a cyclin involved in cell cycle regulation. Overexpression of CCND1 is associated with increased cell proliferation and has been observed in SOCC
- PTEN (Phosphatase and Tensin Homolog): PTEN is a tumor suppressor gene that regulates cell survival and growth. Loss of PTEN function through mutations or deletions is found in OSCC
- MMPs (Matrix Metalloproteinases): MMPs are enzymes that degrade the extracellular matrix and play a role in tumor invasion and metastasis. MMP2 and MMP9 are commonly upregulated in OSCC
- Bcl-2 Family: Members of the Bcl-2 family, including Bcl-2 and Bax, regulate apoptosis. Dysregulation of the Bcl-2 family proteins is implicated in OSCC development and resistance to therapy
- To identify the common gene targets between Squamous Oral Cell Carcinoma (OSCC) and Protein Kinase C (PKC), a comprehensive analysis of the literature and research studies is require

Network and Interaction Analysis

PPI analysis revealed EGFR, ERBB2, HRAS, KRAS and PIK3CA as key hubs, indicating their pivotal role in OSCC (Fig. 2, Table 3).

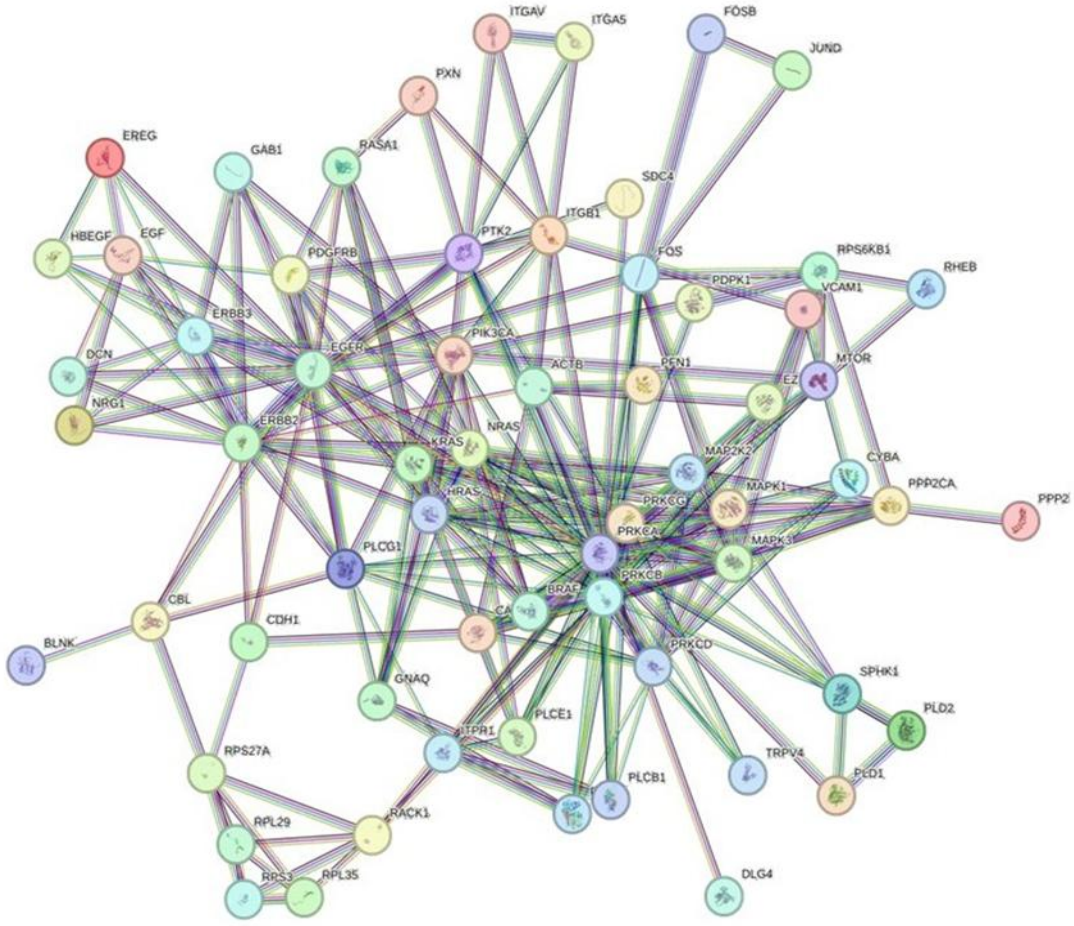

Figure 2: Protein-protein interaction network for common genes retrieved from string database.

Gene Name	Degree	Average Shortest Path Length	Betweenness Centrality	Closeness Centrality	Topological Coefficient
ERBB2	6	2.70	0.03	0.37	0.35
FOS	4	5.07	0.13	0.20	0.50
PRKCD	0	0.00	0.00	0.00	0.00
JUND	2	6.00	0.00	0.17	0.75
PRKCB	0	0.00	0.00	0.00	0.00
EGF	4	2.93	0.00	0.34	0.47
EREG	1	3.03	0.00	0.33	0.00
PLCB4	0	0.00	0.00	0.00	0.00
MAPK3	3	4.27	0.09	0.23	0.56
FOSB	2	6.00	0.00	0.17	0.75
DCN	1	3.03	0.00	0.33	0.00
MTOR	2	1.00	1.00	1.00	0.00
CDH1	1	3.03	0.00	0.33	0.00
RPS3	4	1.00	0.00	1.00	1.00
HRAS	5	2.43	0.17	0.41	0.33
SDC4	1	1.00	0.00	1.00	0.00
GNAQ	1	1.00	0.00	1.00	0.00
BLNK	0	0.00	0.00	0.00	0.00
CFTR	0	0.00	0.00	0.00	0.00
RACK1	4	1.00	0.00	1.00	1.00
ITGB1	4	3.43	0.19	0.29	0.25
RPL29	4	1.00	0.00	1.00	1.00
DLG4	0	0.00	0.00	0.00	0.00
ITGA5	1	4.40	0.00	0.23	0.00
MAPK1	3	4.27	0.09	0.23	0.56
KRAS	4	2.47	0.11	0.41	0.40
PRKCG	0	0.00	0.00	0.00	0.00
RHEB	1	1.50	0.00	0.67	0.00
MAP2K2	3	3.53	0.29	0.28	0.50
EGFR	17	2.07	0.72	0.48	0.18
NRAS	3	2.53	0.11	0.39	0.48
RPS6KB1	1	1.50	0.00	0.67	0.00
EZR	0	0.00	0.00	0.00	0.00
TRPV4	0	0.00	0.00	0.00	0.00
RPL35	4	1.00	0.00	1.00	1.00
PTK2	4	2.67	0.30	0.38	0.33
GAB1	1	3.03	0.00	0.33	0.00
BRAF	4	2.90	0.33	0.34	0.46
PDPK1	0	0.00	0.00	0.00	0.00
PPP2R5A	1	1.00	0.00	1.00	0.00
PLCB1	1	1.00	0.00	1.00	0.00
ITGAV	1	4.40	0.00	0.23	0.00
PLD2	0	0.00	0.00	0.00	0.00
PRKCA	1	1.00	0.00	1.00	0.00

PIK3CA	6	2.60	0.03	0.38	0.36
CYBA	0	0.00	0.00	0.00	0.00
SPHK1	0	0.00	0.00	0.00	0.00
PLCG1	2	3.00	0.00	0.33	0.56
ERBB3	6	2.87	0.01	0.35	0.38
RASA1	1	3.40	0.00	0.29	0.00
PPP2CA	1	1.00	0.00	1.00	0.00
CBL	1	3.03	0.00	0.33	0.00
ACTB	1	1.00	0.00	1.00	0.00
HBEGF	3	2.97	0.00	0.34	0.53
PLCE1	0	0.00	0.00	0.00	0.00
VCAM1	1	4.40	0.00	0.23	0.00
ITPR1	0	0.00	0.00	0.00	0.00
RPS27A	4	1.00	0.00	1.00	1.00
NRG1	3	2.97	0.00	0.34	0.57
PLD1	0	0.00	0.00	0.00	0.00
PXN	1	3.63	0.00	0.28	0.00
PDGFRB	2	3.00	0.00	0.33	0.56
PFN1	1	1.00	0.00	1.00	0.00
CASP3	0	0.00	0.00	0.00	0.00

Table 3: Network Analysis (Degree, Avg path Length, Centrality, Topological Coeff).

Interpretation

ERBB2 emerges as a noteworthy player with a high degree of connectivity, signifying its involvement in multiple interactions within the network. Despite its centrality, ERBB2 does not play a crucial role in connecting different parts of the network, as indicated by its low betweenness centrality. On the other hand, EGFR stands out as a central hub with a remarkably large degree, underscoring its pivotal role in the network. Its high between centrality and closeness centrality suggest that EGFR acts as a crucial connector, efficiently bridging different regions of the network. PIK3CA, while moderately connected, demonstrates efficiency in communication and its neighbours tend to be interconnected, as indicated by the relatively high topological coefficient. In contrast, RPS6KB1 exhibits low connectivity but efficient connections, showcasing its proximity to other nodes without acting as a central hub. This detailed analysis provides nuanced insights into the network dynamics of these genes, guiding further exploration of their functional significance in the context of OSCC and PKC pathways.

Network Topology and Gene Ontology

Network topology showed EGFR had the highest degree of connectivity. Gene ontology indicated involvement in cell signalling, apoptosis regulation, metabolic pathways and tumorigenesis (Fig. 3-5,Table 4-6).

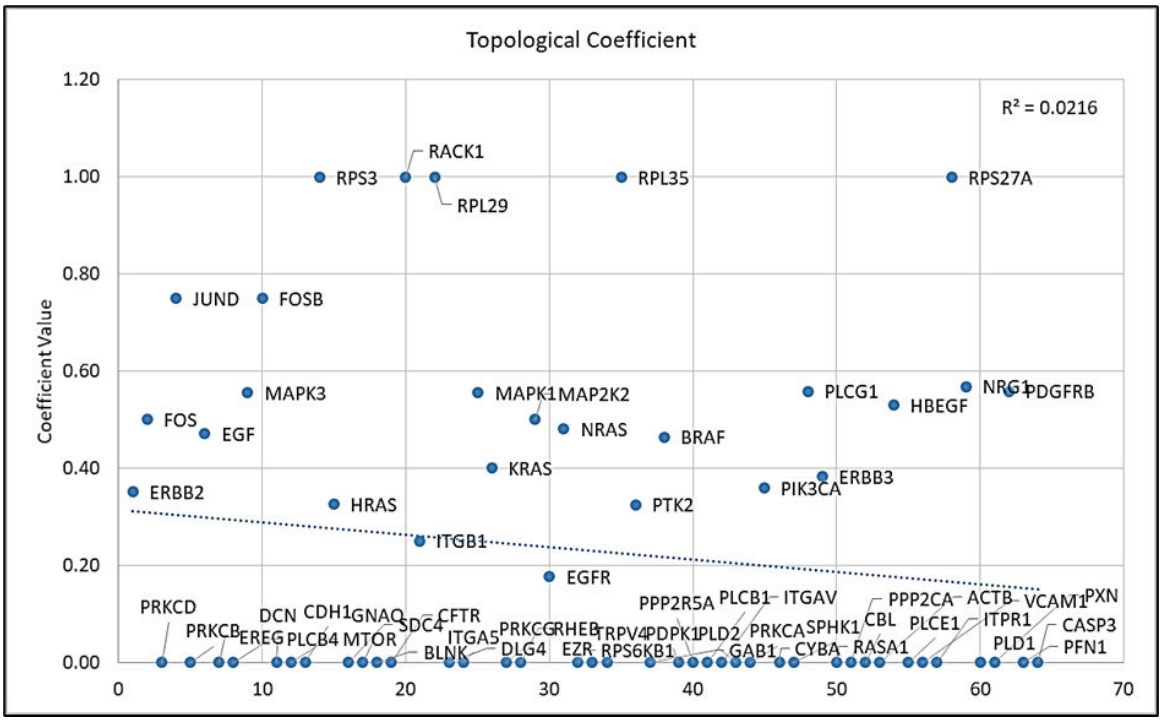

Figure 3: Topological coefficient of the common gene targets between OSCC and PKC.

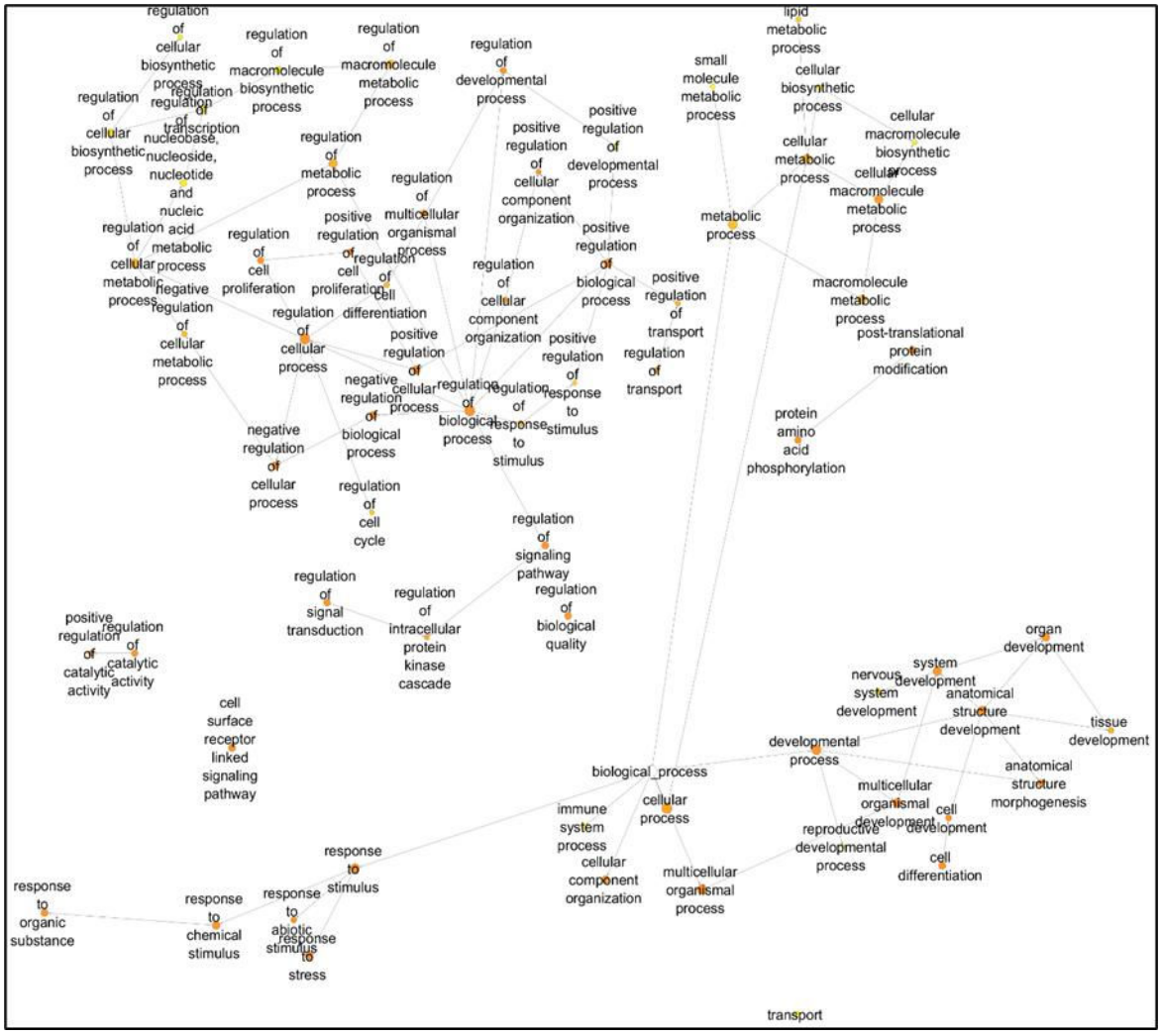

Figure 4: Gene ontology analysis of the common gene targets between OSCC and PKC.

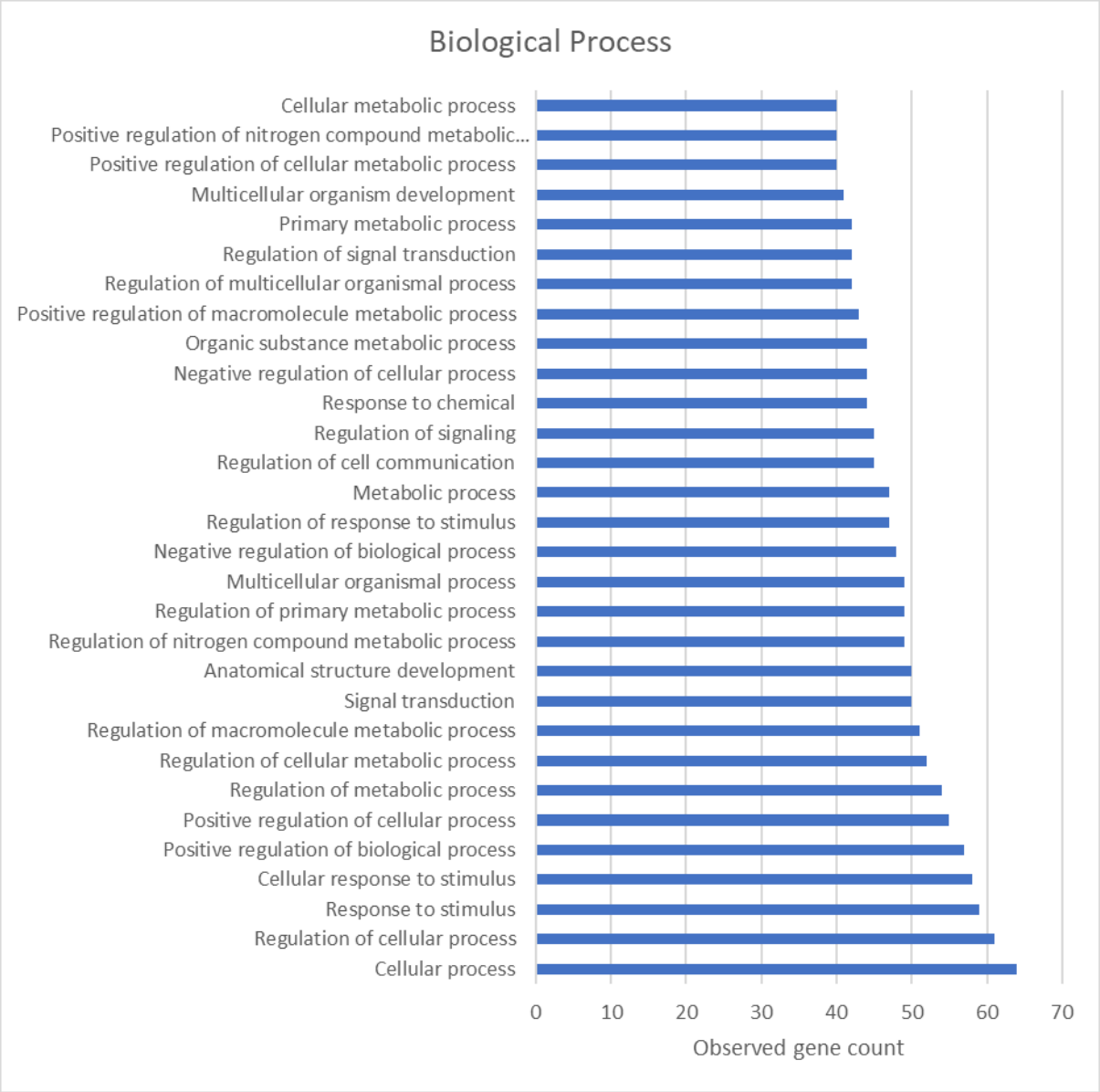

Figure 5: Biological process.

Term ID	Term Description	Gene Count
GO:0009987	Cellular process	64
GO:0050794	Regulation of cellular process	61
GO:0050896	Response to stimulus	59
GO:0051716	Cellular response to stimulus	58
GO:0048518	Positive regulation of biological process	57
GO:0048522	Positive regulation of cellular process	55
GO:0019222	Regulation of metabolic process	54
GO:0031323	Regulation of cellular metabolic process	52
GO:0060255	Regulation of macromolecule metabolic process	51
GO:0007165	Signal transduction	50
GO:0048856	Anatomical structure development	50
GO:0051171	Regulation of nitrogen compound metabolic process	49
GO:0080090	Regulation of primary metabolic process	49
GO:0032501	Multicellular organismal process	49

GO:0048519	Negative regulation of biological process	48
GO:0048583	Regulation of response to stimulus	47
GO:0008152	Metabolic process	47
GO:0010646	Regulation of cell communication	45
GO:0023051	Regulation of signaling	45
GO:0042221	Response to chemical	44
GO:0048523	Negative regulation of cellular process	44
GO:0071704	Organic substance metabolic process	44
GO:0010604	Positive regulation of macromolecule metabolic process	43
GO:0051239	Regulation of multicellular organismal process	42
GO:0009966	Regulation of signal transduction	42
GO:0044238	Primary metabolic process	42
GO:0007275	Multicellular organism development	41
GO:0031325	Positive regulation of cellular metabolic process	40
GO:0051173	Positive regulation of nitrogen compound metabolic process	40
GO:0044237	Cellular metabolic process	40

Table 4: Biological process.

Term ID	Term Description	Gene Count
GO:0005488	Binding	63
GO:0005515	Protein binding	55
GO:0043167	Ion binding	42
GO:0019899	Enzyme binding	38
GO:0003824	Catalytic activity	38
GO:1901363	Heterocyclic compound binding	38
GO:0097159	Organic cyclic compound binding	38
GO:0097367	Carbohydrate derivative binding	29
GO:0043168	Anion binding	29
GO:0036094	Small molecule binding	28
GO:0017076	Purine nucleotide binding	27
GO:0035639	Purine ribonucleoside triphosphate binding	26
GO:0032555	Purine ribonucleotide binding	26
GO:0098772	Molecular function regulator activity	24
GO:0005102	Signaling receptor binding	23
GO:0030554	Adenyl nucleotide binding	22
GO:0019900	Kinase binding	21
GO:0044877	Protein-containing complex binding	21
GO:0005524	ATP binding	21
GO:0140096	Catalytic activity, acting on a protein	21
GO:0019901	Protein kinase binding	19
GO:0016740	Transferase activity	19
GO:0016773	Phosphotransferase activity, alcohol group as acceptor	18
GO:0016301	Kinase activity	18
GO:0016787	Hydrolase activity	18
GO:0004672	Protein kinase activity	17
GO:0030234	Enzyme regulator activity	17
GO:0042802	Identical protein binding	17

Table 5: Molecular function.

Term ID	Term Description	Gene Count
GO:0005622	Intracellular anatomical structure	63
GO:0005737	Cytoplasm	61
GO:0016020	Membrane	59
GO:0043229	Intracellular organelle	59
GO:0043231	Intracellular membrane-bounded organelle	57
GO:0071944	Cell periphery	52
GO:0005886	Plasma membrane	50
GO:0005829	Cytosol	43
GO:0030054	Cell junction	39
GO:0031982	Vesicle	38
GO:0005634	Nucleus	38
GO:0012505	Endomembrane system	37
GO:0032991	Protein-containing complex	36
GO:0042995	Cell projection	31
GO:0120025	Plasma membrane bounded cell projection	30
GO:0070161	Anchoring junction	28
GO:0005576	Extracellular region	28
GO:0031410	Cytoplasmic vesicle	27
GO:0031090	Organelle membrane	27
GO:0005615	Extracellular space	26
GO:0045202	Synapse	24
GO:0005783	Endoplasmic reticulum	23
GO:0098588	Bounding membrane of organelle	23
GO:0098590	Plasma membrane region	21
GO:0005925	Focal adhesion	20
GO:0005794	Golgi apparatus	20
GO:0070062	Extracellular exosome	20

Table 6: Cellular component.

Interpretation of Biological Process

The most significant biological process identified is the general "Cellular process" (GO:0009987), with 64 observed gene counts. This suggests a substantial involvement of these genes in fundamental cellular activities, indicating a potential central role in Oral Squamous Cell Carcinoma (OSCC) and Protein Kinase C (PKC) pathways. Another crucial aspect is the "Regulation of cellular process" (GO:0050794), with 61 observed gene counts. This highlights the importance of fine-tuned control over cellular activities, indicating that the identified genes play a significant role in modulating key cellular processes relevant to OSCC and PKC. A noteworthy finding is the involvement of 59 genes in the "Response to stimulus" process (GO:0050896). This underscores the sensitivity of these genes to external cues, possibly playing a crucial role in how OSCC and PKC respond to environmental signals (Fig. 6,7).

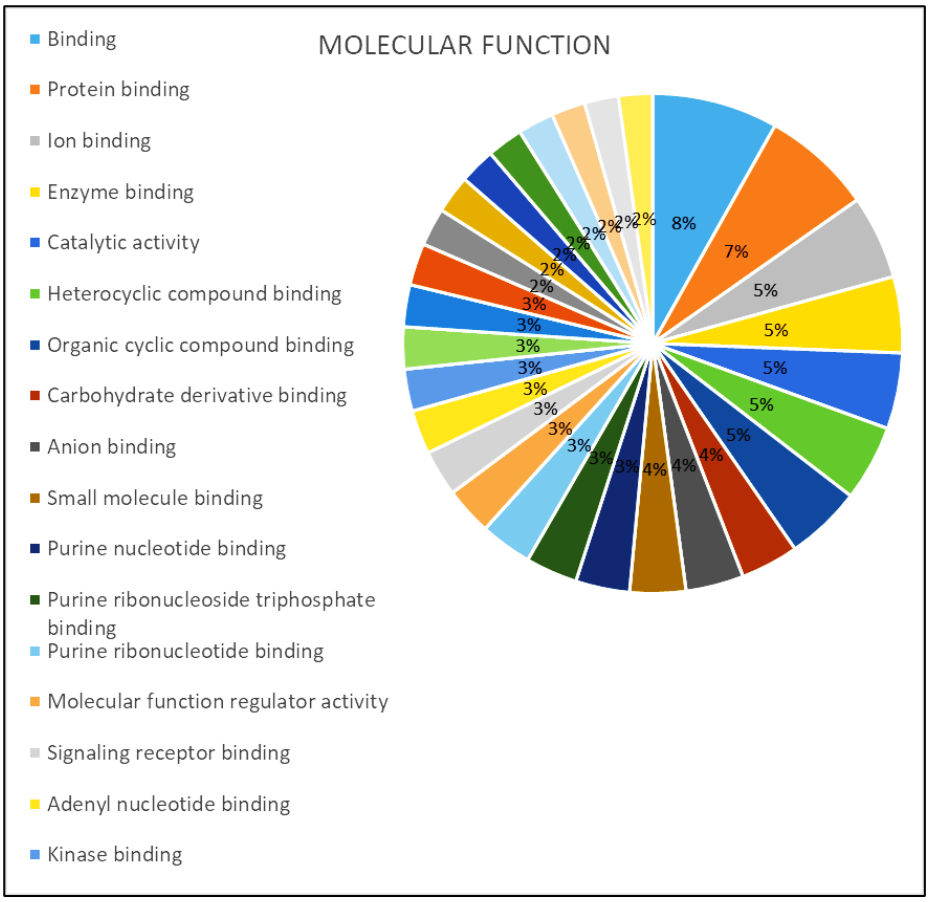

Figure 6: Molecular function.

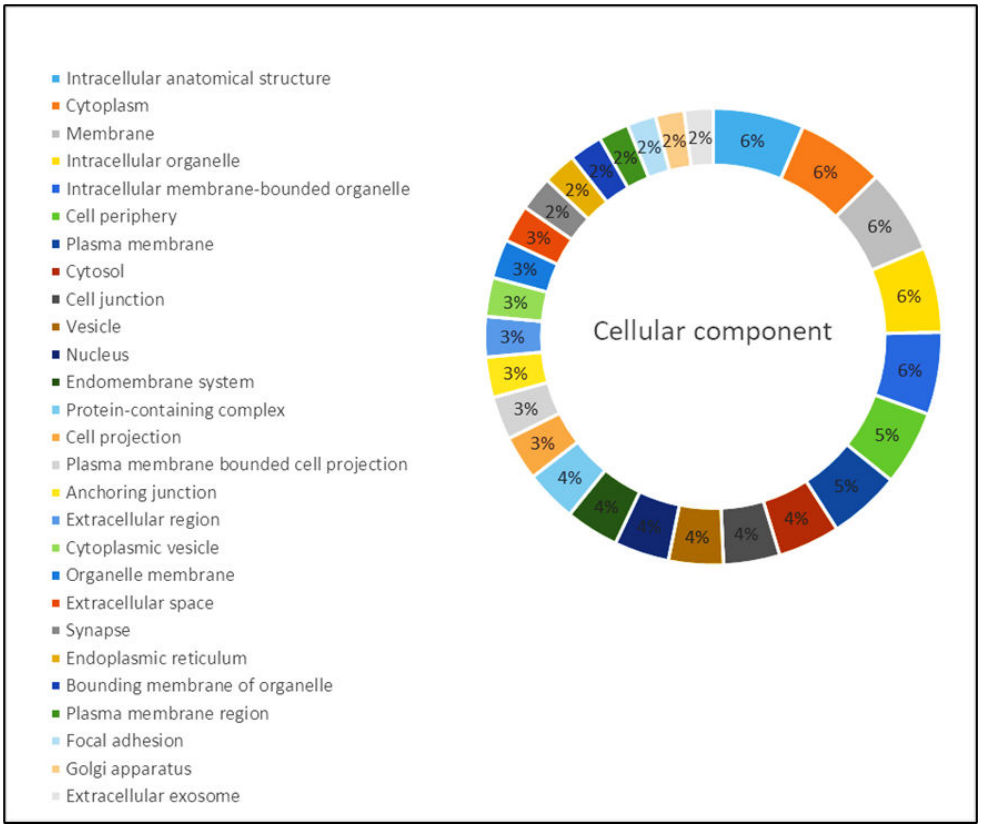

Figure 7: Cellular component.

Interpretation of Molecular Function

The most enriched molecular function is "Binding" (GO:0005488) with 63 observed gene counts. This encompasses a broad category of molecular interactions and suggests that the identified genes are involved in binding to various molecular entities, indicating their versatile roles in cellular processes. "Protein binding" (GO:0005515) is another prominent molecular function with 55 observed gene counts. This implies that a substantial number of genes in the dataset are involved in protein-protein interactions, indicating their significance in forming complexes and regulating biological pathways. Notably, there is enrichment in catalytic activities, including "Catalytic activity" (GO:0003824) and "Enzyme binding" (GO:0019899), both with 38 observed gene counts. This suggests a significant involvement of the identified genes in enzymatic processes, underscoring their potential roles as catalysts in biochemical reactions.

Interpretation for Cellular Component

The most enriched category is "Intracellular anatomical structure" (GO:0005622) with 63 observed gene counts. This indicates a significant presence of genes associated with internal cellular structures, suggesting their involvement in intracellular processes crucial for Oral Squamous Cell Carcinoma (OSCC) and Protein Kinase C (PKC)."Cytoplasm" (GO:0005737) and "Intracellular organelle" (GO:0043229) with 61 and 59 observed gene counts, respectively, highlight the importance of cytoplasmic processes and intracellular organelles. This suggests that the identified genes play key roles in cellular functions within these compartments. The presence of "Membrane" (GO:0016020) and "Intracellular membrane-bounded organelle" (GO:0043231) with 59 and 57 observed gene counts, respectively, indicates a strong association with membrane-related activities. This includes processes such as signalling, transport and compartmentalization, which are crucial for OSCC and PKC pathways.

In summary, these findings provide insights into the cellular localization and functions of the genes associated with OSCC and PKC, offering potential avenues for further research and therapeutic targeting.

Pan-Drug Analysis

Interpretation

In the PAN Drug Analysis of common target genes associated with Oral Squamous Cell Carcinoma (OSCC) and Protein Kinase C (PKC), a comprehensive understanding of potential therapeutic interventions emerges. Several key genes exhibit associations with specific drugs, each with a DScore indicating the strength of the drug-gene interaction:

- Notably, CETUXIMAB , with a DScore of 1, emerges as a promising candidate. This drug targets a cluster of genes including BRAF, EGFR, ERBB2, HRAS, KRAS, PIK3CA and PRKCA, suggesting a multifaceted approach to treatment
- Notably, tabulated drugs have the potential to address the complexity of OSCC and PKC networks, providing valuable insights for personalized therapeutic strategies
- The high DScores underscore the significance of these drugs in potentially modulating the dysregulated pathways associated with OSCC and PKC, warranting further investigation and consideration in clinical contexts
- These drug-gene associations provide valuable insights into potential therapeutic strategies for OSCC, indicating drugs that may have specific interactions with the identified target genes. Finally, CETUXIMAB which has the highest score and the greatest number of protein interaction including PKC was selected for the molecular docking analysis (Fig. 8,Table 7)

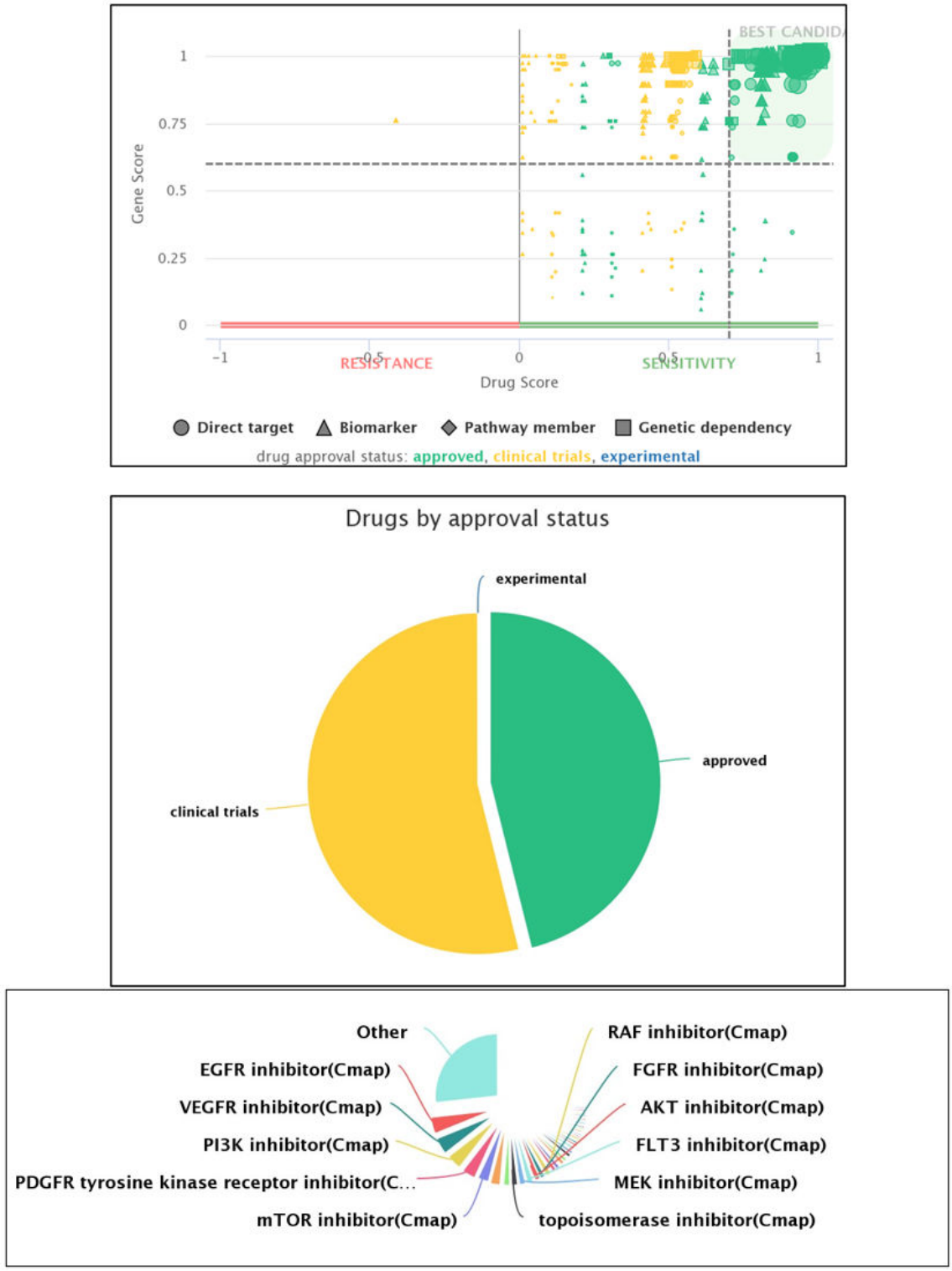

Figure 8: Representation of gene and drug score, drug family.

Gene Names	Drug Name	DScore
BRAF|EGF|EGFR|ERBB2|EREG|HBEGF|HRAS|KRAS|NRAS|NRG1|PIK3CA	CETUXIMAB	1
BRAF|EGFR|ERBB2|GNAQ|HRAS|KRAS|MTOR|NRAS|PIK3CA	EVEROLIMUS	1
BRAF|EGF|EGFR|ERBB2|EREG|HBEGF|HRAS|KRAS|NRAS|NRG1	PANITUMUMAB	1
BRAF|EGFR|ERBB2|KRAS|MAPK1|MAPK3|NRAS	SORAFENIB	1

|PDGFRB|PIK3CA|RPS6KB1		
BRAF|EGFR|GNAQ|HRAS|KRAS|MAP2K2|NRAS|PIK3CA	TRAMETINIB	0.99
BRAF|ERBB2|HRAS|KRAS|MTOR|NRAS|PIK3CA	ALPELISIB	0.98
BRAF|CBL|CDH1|EGFR|ERBB2|ERBB3|KRAS	ERLOTINIB	0.98
BRAF|CDH1|EGFR|ERBB2|ERBB3|KRAS|PIK3CA	LAPATINIB	0.98
EGFR|ERBB2|ERBB3|KRAS|MAP2K2|MTOR|PDGFRB	VANDETANIB	0.98
BRAF|EGFR|ERBB2|KRAS|NRAS|PIK3CA	AFATINIB	0.97
BRAF|EGFR|HRAS|KRAS|NRAS|PIK3CA	DABRAFENIB	0.97
BRAF|EGFR|ERBB2|ERBB3|KRAS|NRAS	GEFITINIB	0.97
BRAF|EGFR|ERBB2|ERBB3|KRAS|PIK3CA	NERATINIB	0.97
BRAF|EGFR|ERBB2|KRAS|MTOR|PIK3CA	TEMSIROLIMUS	0.97
BRAF|EGFR|ERBB2|ERBB3|KRAS|PIK3CA	TRASTUZUMAB	0.97
BRAF|EGFR|HRAS|KRAS|NRAS|PIK3CA	VEMURAFENIB	0.97
BRAF|GNAQ|HRAS|KRAS|MAP2K2|NRAS	BINIMETINIB	0.968
BRAF|EGFR|KRAS|NRAS|PDGFRB|PIK3CA	REGORAFENIB	0.967
ERBB2|NRG1|PIK3CA|PLD1|PLD2|PRKCA	TAMOXIFEN	0.962
BRAF|KRAS|MAP2K2|NRAS|PIK3CA	COBIMETINIB	0.96
EGFR|ERBB2|ERBB3|PIK3CA	DACOMITINIB	0.95
EGFR|ERBB2|KRAS|NRAS	MERELETINIB	0.95

Table 7: PAN drug analysis of the common gene targets of OSCC and PKC.

Molecular Docking

The binding energy and bond length of each interaction has been mentioned in Table 10. Docking results were integrated with network and pharmacological data to identify the most promising compounds. These findings suggest that the CETUXIMAB has a strong binding affinity for PRKCA, as indicated by the favourable binding energy. The presence of multiple hydrogen bonds, both conventional and carbon hydrogen bonds, indicates specific and varied interactions between the ligand and PRKCA (Fig. 9-15,Table 8-11). Docking revealed three key hydrogen bonds, Conventional H-bonds with Val A:420 (3.22 Å), Val A:353 (4.98 Å) and Carbon H-bond with Asp A:481 (3.53 Å). These interactions suggest a stable and specific binding, reinforcing CETUXIMAB potential as a targeted therapy for OSCC. Therefore, it can be clearly stated that CETUXIMAB can be effectively repurposed as drug for the treatment of oral squamous cell carcinoma targeting the Active residue pockets of Protein Kinase C.

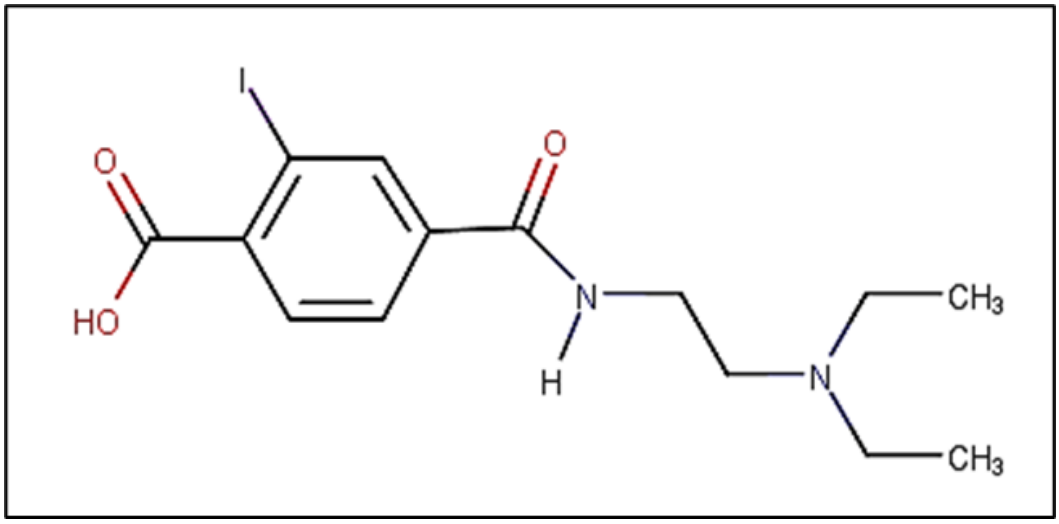

Figure 9: Two-dimensional structure of CETUXIMAB.

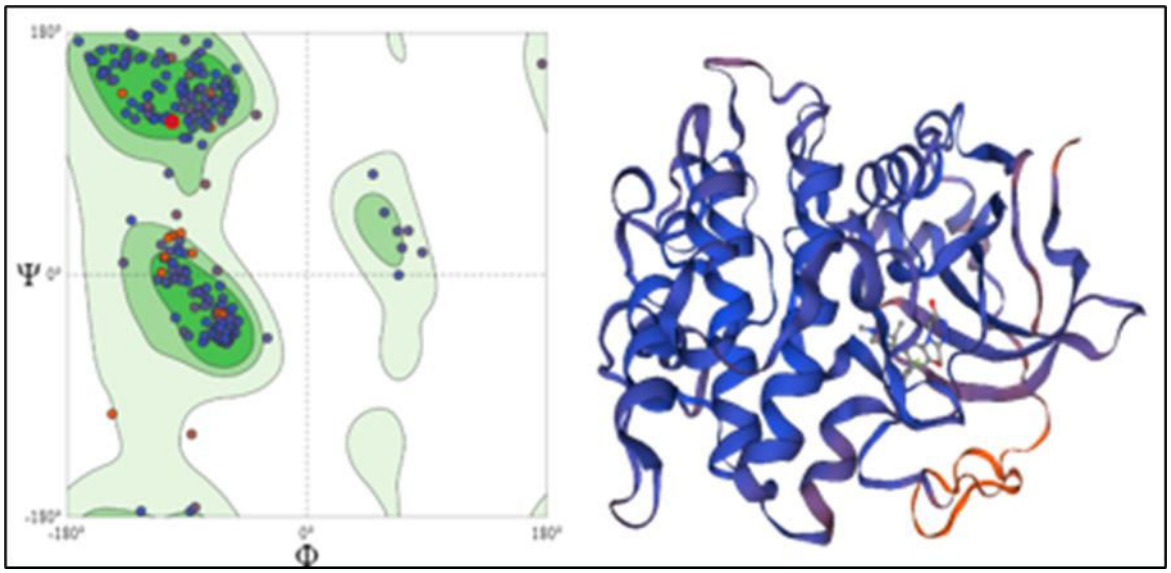

Figure 10: Ramachandran Plot and 3D structure of PKC.

```
+----------<<<  P  R  O  C  H  E  C  K     S  U  M  M  A  R  Y  >>>----------+
|                                                                             |
|  /var/www/SAVES/Jobs/1474454/saves.pdb   1.5                  335 residues |
|                                                                             |
+|  Ramachandran plot:   91.4% core    7.2% allow    1.4% gener    0.0% disall |
|                                                                             |
+|  All Ramachandrans:    7 labelled residues (out of 333)                     |
+|  Chi1-chi2 plots:      2 labelled residues (out of 218)                     |
   Side-chain params:    5 better     0 inside      0 worse                    |
                                                                              |
+|  Residue properties: Max.deviation:     4.2             Bad contacts:    0  |
+|                      Bond len/angle:    4.9    Morris et al class:  1  2  2 |
                                                                              |
   G-factors           Dihedrals:  -0.18  Covalent:  -0.01    Overall:  -0.10 |
                                                                              |
+|  Planar groups:    91.0% within limits   9.0% highlighted       2 off graph |
                                                                              |
 +-----------------------------------------------------------------------------+
   + May be worth investigating further.  * Worth investigating further.
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Figure 11: Procheck summary of Protein Kinase C alpha.

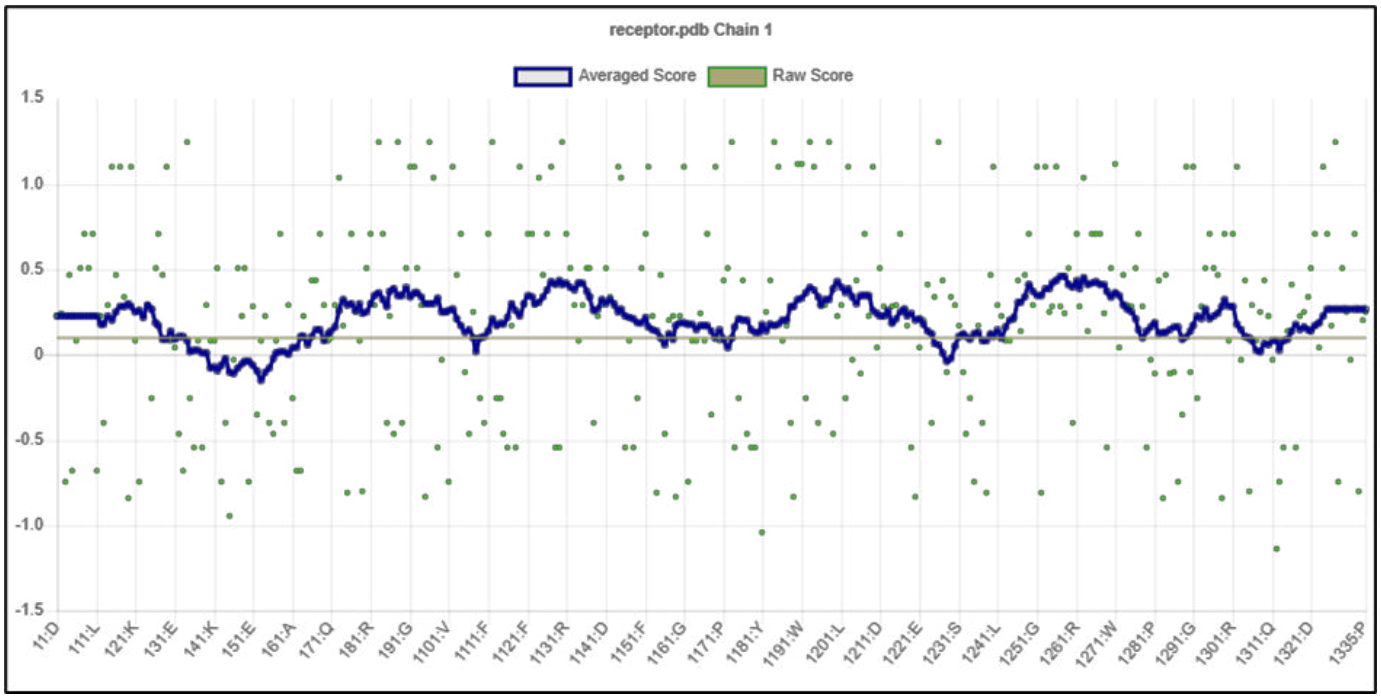

Figure 12: Verify3D of Protein kinase C alpha.

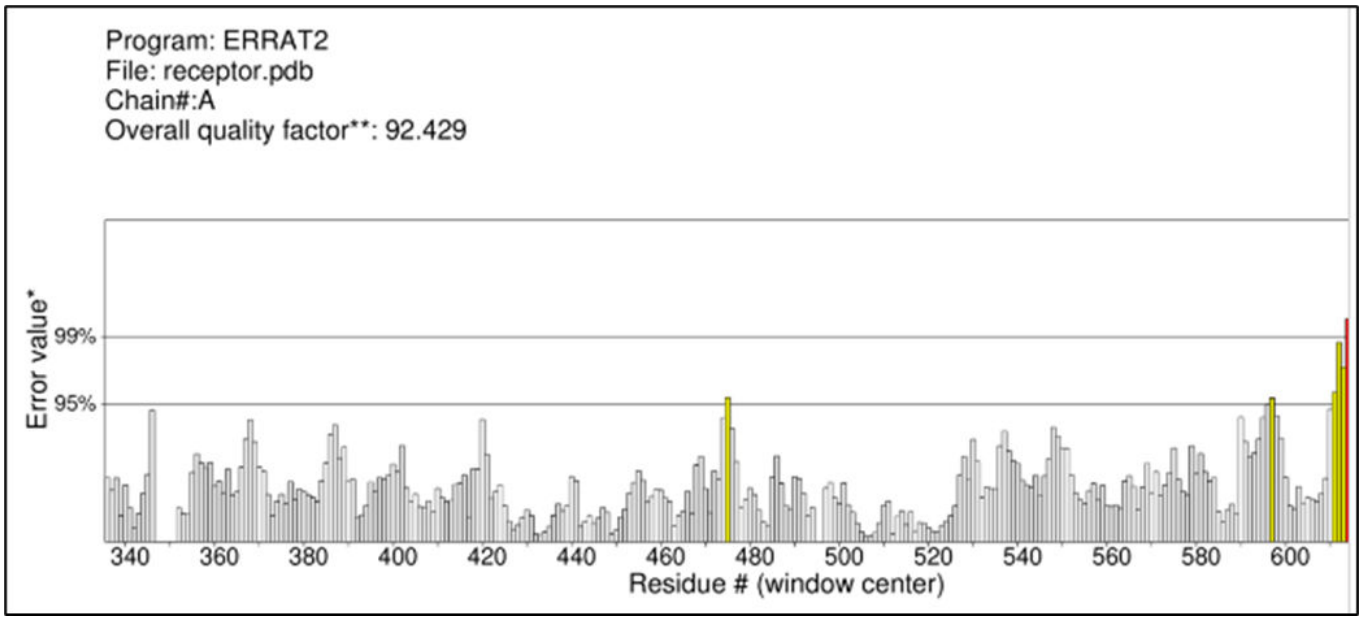

Figure 13: ERRAT of Protein Kinase C alpha.

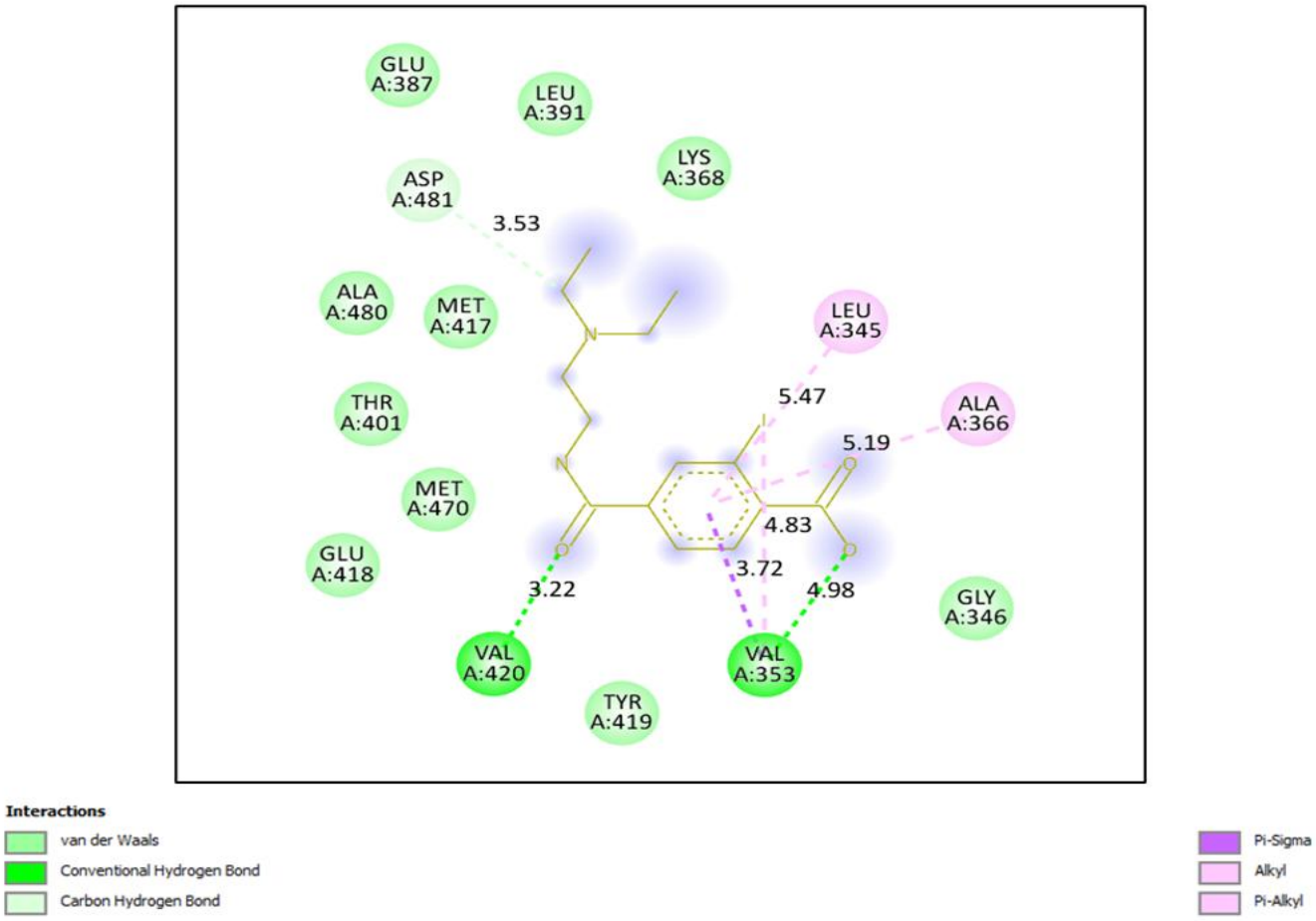

Figure 14: 2D interaction of Protein kinase C alpha with CETUXIMAB.

S.No	Uniprot ID	Target Protein Name
1	P17252	Protein Kinase C

Table 8: Uniprot ID of selected target protein.

Target	Uniprot ID	Protein Name	Ramachandran flavoured (%)	Clash score	Qmean
PRKCA	P17252	Protein Kinase C Alpha	94.59	0.37	0.81

Table 9: Ramachandran plot and clash score.

Protein Name	ERRAT Quality Score	Procheck	Verify 3D
PRKCA	92.429	Out of 8 evaluations, Errors: 0 Warning: 6 Pass: 2	82.39 % of the residues have average 3D-1D score of >= 0.1

Table 10: Qality Score of three-dimensional protein modelling of PKC.

S.No	Targets	Binding energy (Kcal/mol)	No of Hydrogen bonds	H-Bond with bond length	
				Conventional H-Bond	Carbon hydrogen bond
1	PRKCA	-5.9	3	Val A:420 (3.22Å), Val A: 353 (4.98 Å)	Asp A: 481 (3.53 Å)

Table 11: Binding energy and bond length of metformin with target proteins.

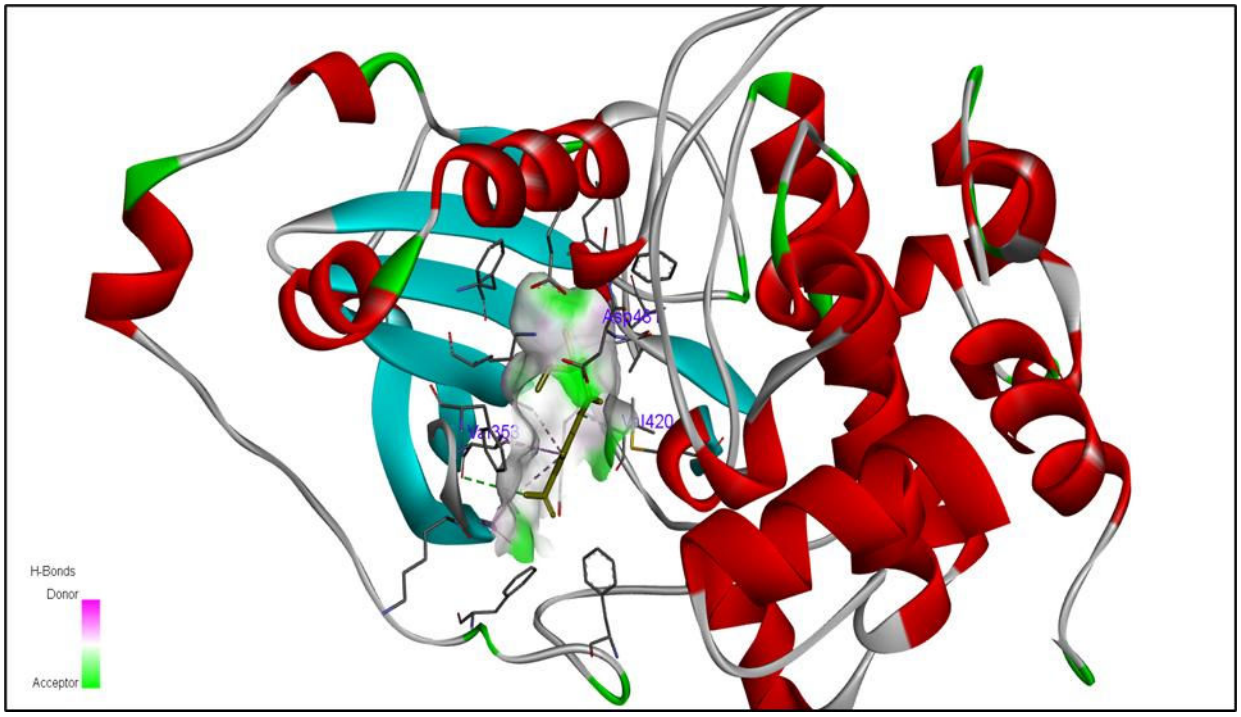

Figure 15: 3D interaction of Protein kinase C alpha with CETUXIMAB.

Discussion

The present In-silico study explored the Protein-Protein Interaction (PPI) network of Protein Kinase C (PKC) in the context of Oral Squamous Cell Carcinoma (OSCC), followed by molecular docking and pan-drug analysis to identify potential therapeutic targets. PKC has been extensively implicated in tumor initiation, progression, angiogenesis and metastasis due to its role in regulating cell proliferation, survival and invasion. Our network topology analysis highlighted PKC as a central hub, reinforcing its importance in OSCC pathobiology.

Molecular docking analysis demonstrated significant interactions of PKC with CETUXIMAB, a chimeric monoclonal antibody that targets the epidermal growth factor receptor (EGFR). CETUXIMAB binding in our in-silico model suggests that PKC-related signaling may be indirectly modulated through EGFR inhibition. This aligns with previous reports that EGFR is frequently overexpressed in OSCC and its blockade reduces tumor growth and angiogenesis. Thus, the computational findings of our study are consistent with existing evidence, while further highlighting the therapeutic relevance of CETUXIMAB in the PKC-centered network.

Pan-drug analysis further supported the robustness of CETUXIMAB as a candidate, suggesting broad-spectrum potential within OSCC therapeutic frameworks. Importantly, CETUXIMAB is already clinically approved for head and neck squamous cell carcinoma which strengthens the translational significance of our findings. The ability of our computational pipeline to identify a clinically validated agent underscores its reliability and applicability in guiding drug repurposing efforts.

The network and docking analyses provide strong preliminary insights, they cannot fully replicate the complexity of tumor microenvironments, drug metabolism and interpatient variability. Further in vitro and in vivo validation is essential to confirm the precise role of PKC-EGFR enhance the efficacy of Cetuximab in OSCC-specific contexts.

In summary, this study highlights PKC as a critical hub in OSCC and identifies CETUXIMAB as a promising therapeutic candidate through computational drug repurposing. Our findings not only support the current role of EGFR-targeted therapies in OSCC but also provide a framework for future experimental validation and the development of integrated therapeutic strategies.

Conclusion

A total of 4945 genes associated with Oral Squamous Cell Carcinoma (OSCC) and 101 PKC-interacting genes were identified, with 64 common targets. These were further examined through Protein-Protein Interaction (PPI) network, gene ontology and pan-drug analysis. Key central nodes in the PPI network included EGFR, ERBB2, HRAS, KRAS and PIK3CA, with EGFR showing the highest connectivity. Gene ontology analysis indicated significant roles in cellular signalling, apoptosis regulation, metabolism and tumorigenesis. Pan-drug analysis identified CETUXIMAB as the top candidate (DScore 0.872), targeting BRAF, EGFR, ERBB2, HRAS, KRAS, PIK3CA and PRKCA. Additional drugs such as CARBOPLATIN, PACLITAXEL, DOCETAXEL, DOXORUBICIN and CISPLATIN also showed high potential. Molecular docking revealed that CETUXIMAB binds PKC Alpha (PRKCA) with -5.9 kcal/mol binding energy, forming hydrogen bonds with Val A:420, Val A:353 and Asp A:481, indicating strong and specific interaction.

Conflict of Interest Statement

All authors declare that there are no conflicts of interest.

Informed Consent Statement

Informed consent was taken for this study.

Authors' Contributions

All authors contributed equally to this paper.

Financial Disclosure

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Data Availability Statement

Not applicable.

Ethical Statement

Not applicable.

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