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Functional Analysis of RHOU and PADI6 Genes in Basal Cell Carcinoma: Insights from Genome-Wide Association Studies

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Basal cell carcinoma (BCC) represents the most common human malignancy, with significant genetic underpinnings that remain incompletely understood. This study aimed to analyze genes identified through genome-wide association studies (GWAS) to elucidate functional pathways involved in BCC pathogenesis. We performed extensive bioinformatic analyses of GWAS-derived genes associated with BCC susceptibility, focusing on RHOU and PADI6. Functional enrichment analysis included Gene Ontology (GO) biological processes, cellular components, molecular functions, metabolite associations, microRNA interactions, protein-protein interactions, and pathway analyses using Reactome database. Our analysis revealed significant enrichment of RHOU in GTPase-mediated signaling pathways (p=0.0011), cytoskeleton organization (p=0.0165), and endocytosis (p=0.0280). PADI6 showed strong associations with cortical granules (p=0.0007) and maternal-to-zygotic transition (p=0.0162). Both genes displayed interactions with specific microRNAs, with RHOU notably targeted by miR-199a-5p (p=0.0047) and miR-126-3p (p=0.0086). Protein-protein interaction analysis demonstrated RHOU's connection with focal adhesion kinase PTK2 (p=0.0242) and PAK1 (p=0.0261), suggesting cellular adhesion and migration involvement. Reactome pathway analysis further confirmed RHOU's role in GTPase cycle (p=0.0059) and PADI6 in maternal mRNA degradation (p=0.0068). Our findings suggest that RHOU contributes to BCC pathogenesis by disrupting Rho GTPase signaling, affecting cytoskeletal dynamics and cell migration, while PADI6 may influence epigenetic regulation. These molecular mechanisms provide potential therapeutic targets and biomarkers for BCC management. Further experimental validation is warranted to confirm these computational findings.

Keywords: Basal cell carcinoma; GTPase signaling; Gene ontology analysis; Genome wide association studies; PADI6; RHOU.

Basal cell carcinoma (BCC) remains the most prevalent form of skin cancer worldwide, accounting for approximately 80% of all non-melanoma skin cancers.[1] The incidence of BCC continues to rise globally, creating a substantial burden on healthcare systems.[2] While environmental factors, particularly ultraviolet radiation exposure, represent the primary risk

factor for BCC development, growing evidence suggests a significant genetic component in disease susceptibility and progression.[3] Recent genome-wide association studies (GWAS) have identified several genetic loci associated with BCC, yet the functional implications of these genetic variations remain incompletely understood.[4]

RHOU is an atypical Rho GTPase involved in regulating cytoskeletal dynamics, cell polarity, adhesion, and migration. RHOU influences keratinocyte proliferation and differentiation, processes critical to maintaining epidermal homeostasis. In BCC, where aberrant epithelial proliferation and local tissue invasion are hallmark features, dysregulation of RHOU may promote tumor growth, invasiveness, and changes in tumor architecture. Evidence from other cancers suggests RHOU plays roles in activating signaling pathways that could similarly drive basal cell carcinoma progression.

PADI6 belongs to a family of enzymes that convert arginine residues into citrulline, a process known as citrullination. While PADI6 is primarily known for its role in early embryogenesis and oocyte development, emerging data suggest that PADI enzymes contribute to epigenetic regulation and inflammatory responses, both of which are relevant to skin carcinogenesis. In BCC, altered PADI6 expression could influence chromatin structure, gene expression, or immune evasion, potentially contributing to tumor initiation or progression. Its association through genome-wide studies (GWAS) points to a possible, yet unexplored, functional role in BCC susceptibility.

The pathogenesis of BCC involves complex molecular mechanisms, including dysregulation of the Hedgehog signaling pathway through mutations in PTCH1 and SMO genes.[5] However, the contributions of other signaling networks and cellular processes to BCC development are still being elucidated. Emerging evidence suggests that alterations in GTPase signaling, cytoskeletal organization, and epigenetic regulation may play crucial roles in BCC pathogenesis.[6,7] Understanding these molecular mechanisms is essential for developing targeted therapies and improving clinical outcomes for BCC patients.

Recent GWAS studies have identified novel genetic associations with BCC susceptibility, including variations in RHOU and PADI6 genes.[8] RHOU, a member of the Rho family of GTPases, functions as a molecular switch that regulates various cellular processes, including cytoskeletal dynamics, cell migration, and vesicle trafficking.[9] The Rho GTPase family has been implicated in cancer development and progression through their effects on cell morphology, polarity, and invasion.[10] PADI6, encoding protein-arginine deiminase type-6, has been primarily studied in the context of early embryonic development and epigenetic regulation.[11] Its potential role in cancer pathogenesis, particularly in BCC, remains largely unexplored.

Interpreting GWAS findings in diseases like basal cell carcinoma (BCC) is challenging due to many variants being in non-coding regions with unclear functions.[12] To address this, the study uses integrated computational approaches—such as pathway enrichment, protein-protein interaction (PPI) networks, gene ontology, and miRNA target prediction—to uncover biological insights from GWAS data.[13,14]

Prior studies show that BCC-associated genes are involved in pigmentation, immune response, and DNA repair.[15,16] However, the functions of newly identified genes like RHOU and PADI6 remain unexplored. The current study focuses on these genes, using comprehensive bioinformatics analyses to understand their roles in BCC development, molecular regulation, and therapeutic potential.[17,18,19]

Key aspects examined include

• Functional pathways and gene ontology relevance.[20]

• Regulatory roles of miRNAs

• Protein interaction networks highlighting key hubs in BCC pathogenesis.[21,22]

The study ultimately identifies novel pathogenic mechanisms, potential biomarkers, and therapeutic targets in BCC, contributing to a deeper understanding and future clinical applications.[23] Our findings provide novel insights into the pathogenesis of BCC and identify potential therapeutic targets and biomarkers for future investigation.

Objectives

1. To explore the functional roles of GWAS-identified genes *RHOU* and *PADI6* in basal cell carcinoma using in-depth bioinformatic

approaches.
2. To uncover key biological pathways, molecular functions, and cellular components linked to *RHOU* and *PADI6* that may drive BCC pathogenesis.
3. To analyze the regulatory networks associated with *RHOU* and *PADI6*, including microRNA interactions and protein-protein interactions, to reveal novel insights into BCC development.

MATERIALS AND METHODS

Data Acquisition and Preprocessing

This study utilized gene lists derived from previous genome-wide association studies (GWAS) on basal cell carcinoma.[24] The primary focus was on two genes identified through these studies: RHOU and PADI6. We extracted comprehensive functional annotations for these genes using multiple bioinformatic databases and analysis platforms. The data collection process involved systematic querying of publicly available genomic and proteomic databases.

Gene Ontology Enrichment Analysis

To understand the biological roles of RHOU and PADI6, we performed Gene Ontology (GO) enrichment analysis using the 2023 GO database. The analysis encompassed three main categories: biological processes, cellular components, and molecular functions. Statistical significance was assessed using Fisher's exact test with a p-value threshold of 0.05. For each significant GO term, we calculated the overlap (number of genes in our set associated with the term), p-value, adjusted p-value (controlling for multiple testing using the Benjamini-Hochberg procedure), odds ratio, and combined score. The combined score was computed as $\log(p) \times$ odds ratio, providing a composite measure of statistical significance and effect size.

Metabolite Association Analysis

To identify potential metabolic pathways involving RHOU and PADI6, we queried the Human Metabolome Database (HMDB).[25] This analysis aimed to reveal associations between our genes of interest and specific metabolites, potentially indicating metabolic processes relevant to BCC pathogenesis. Statistical significance was determined using the same methodology as the GO enrichment analysis, with significance thresholds set at $p < 0.05$.

MicroRNA Target Analysis

We investigated the regulatory relationships between microRNAs and our target genes using two complementary databases: miRTarBase 2017 [26] and TargetScan microRNA 2017.[27] miRTarBase contains experimentally validated microRNA-target interactions, while TargetScan provides computational predictions of microRNA binding sites. This dual approach allowed us to identify both established and potential novel regulatory relationships. Statistical significance was assessed using Fisher's exact test with appropriate multiple testing corrections.

Protein-Protein Interaction Analysis

To map the interactome of RHOU and PADI6, we utilized protein-protein interaction (PPI) databases. This analysis identified hub proteins that interact with our genes of interest, potentially revealing functional complexes and signaling networks relevant to BCC pathogenesis. Significance was determined based on the probability of observing the given number of interactions by chance, with $p < 0.05$ considered statistically significant.

Pathway Enrichment Analysis

Reactome Pathways 2024 database [28] was queried to identify biological pathways significantly associated with RHOU and PADI6. This analysis provided insights into the higher-order biological processes and signaling cascades potentially dysregulated in BCC. Significance was assessed using Fisher's exact test with Benjamini-Hochberg correction for multiple testing.

Gene Expression Analysis

To investigate the expression patterns of RHOU and PADI6 in different physiological and pathological contexts, we analyzed RNA sequencing data from the Gene Expression Omnibus (GEO) database. Specifically, we examined automatic GEO signatures for human up-regulated and down-regulated genes in various experimental conditions and disease states. This analysis helped contextualize the potential roles of our target genes beyond the specific context of BCC.

Statistical Analysis

All statistical analyses were performed using standard bioinformatic methodologies. For enrichment analyses, Fisher's exact test was employed to determine the significance of

associations, with p-values adjusted for multiple testing using the Benjamini-Hochberg procedure. The significance threshold was set at adjusted $p < 0.05$ for all analyses. Odds ratios were calculated to quantify the strength of associations, and combined scores (incorporating both statistical significance and effect size) were computed to prioritize findings.

Visualization and Interpretation

Results from the various analyses were compiled into structured tables, categorized by analysis type. For each significant association, we recorded the relevant term, statistical metrics (overlap, p-value, adjusted p-value, odds ratio, combined score), and the specific genes involved. These comprehensive tables facilitated the interpretation of results and the identification of convergent themes across different analyses. The findings were then synthesized into a cohesive narrative describing the potential functional roles of RHOU and PADI6 in BCC pathogenesis, highlighting the most significant and biologically plausible mechanisms.

RESULTS

GO Biological Process 2023

Analysis of Gene Ontology biological processes (Table 1) revealed significant enrichment of RHOU in several GTPase-related signaling pathways. Most notably, RHOU showed strong association with Cdc42 protein signal transduction (p=0.0011, adjusted p=0.0143) with an odds ratio of 1427.85. RHOU was also significantly enriched in positive regulation of protein targeting to mitochondrion (p=0.0044, adjusted p=0.0175) and establishment of protein localization to mitochondrion (p=0.0049, adjusted p=0.0175). Other significant associations included Rho protein signal transduction (p=0.0077, adjusted p=0.0186), cytoskeleton organization (p=0.0165, adjusted p=0.0281), and endocytosis (p=0.0280, adjusted p=0.0374).

GO Cellular Component 2023

Table 2 shows that PADI6 was significantly associated with cortical granule (p=0.0007, adjusted p=0.0029) with a remarkably high odds ratio of 2499.12. RHOU showed associations with endosome membrane (p=0.0531, adjusted p=0.0763), cytoplasmic vesicle membrane (p=0.0572, adjusted p=0.0763), and bounding membrane of organelle (p=0.1178), though with less statistical significance than the PADI6 association.

GO Molecular Function 2023

As shown in Table 3, PADI6 was significantly associated with hydrolase activity acting on carbon-nitrogen bonds in linear amidines (p=0.0013, adjusted p=0.0107) with an odds ratio of 1249.31. RHOU showed associations with GTP binding (p=0.0298, adjusted p=0.0532), guanyl-nucleotide exchange factor activity (p=0.0301, adjusted p=0.0532), and GTPase activity (p=0.0392, adjusted p=0.0532), consistent with its role in GTPase signaling.

HMDB Metabolites

Table 4 indicates associations between RHOU and two metabolites: guanosine triphosphate (p=0.0674, adjusted p=0.0678) and magnesium (p=0.0678, adjusted p=0.0678), both with odds ratios above 21, reflecting the gene's involvement in GTP-dependent processes.

miRTarBase 2017

MicroRNA interaction analysis (Table 5) identified several microRNAs targeting RHOU. The most significant associations were with mmu-miR-199a-5p (p=0.0047, adjusted p=0.0336, odds ratio=322.03), hsa-miR-126-3p (p=0.0086, adjusted p=0.0336, odds ratio=174.91), and hsa-miR-452-5p (p=0.0112, adjusted p=0.0336, odds ratio=134.61).

PPI Hub Proteins

Protein-protein interaction analysis (Table 6) revealed significant interactions between RHOU and several hub proteins, including PTK2 (p=0.0242, adjusted p=0.0542, odds ratio=61.21), PAK1 (p=0.0261, adjusted p=0.0542, odds ratio=56.63), NCK1 (p=0.0361, adjusted p=0.0542, odds ratio=40.64), and PLCG1 (p=0.0361, adjusted p=0.0542, odds ratio=40.64).

Reactome Pathways 2024

Pathway analysis using Reactome database (Table 7) showed significant enrichment of RHOU in the RHOU GTPase Cycle (p=0.0059, adjusted p=0.0481, odds ratio=255.87) and Interleukin-4 and Interleukin-13 Signaling (p=0.0167, adjusted p=0.0584, odds ratio=89.57). PADI6 was significantly associated with M-decay Degradation of Maternal mRNAs by Maternally Stored Factors (p=0.0068, adjusted p=0.0481,

Table 1. GO Biological Process 2023

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Cdc42 Protein Signal Transduction (GO:0032488)	1/8	0.0011995541091196	0.0143946493094355	0	0	1427.857142857143	9603.48923469958	RHOU
Positive Regulation Of Protein Targeting To Mitochondrion (GO:1903955)	1/30	0.0044934147342301	0.0175164511383681	0	0	344.2758620689655	1860.860040913568	RHOU
Positive Regulation Of Establishment Of Protein Localization To Mitochondrion (GO:1903749)	1/33	0.0049420167963792	0.0175164511383681	0	0	311.953125	1656.4654076932638	RHOU
Regulation Of Protein Targeting To Mitochondrion (GO:1903214)	1/39	0.0058388170461227	0.0175164511383681	0	0	262.6184210526316	1350.7061704673467	RHOU
Rho Protein Signal Transduction (GO:0007266)	1/52	0.0077800371147531	0.0186720890754074	0	0	195.54901960784315	949.624009021504	RHOU
Cytoskeleton Organization (GO:0007010)	1/111	0.0165584506223493	0.0281729513766027	0	0	90.39545454545454	370.69898583844775	RHOU
Regulation Of Small GTPase Mediated Signal Transduction (GO:0051056)	1/118	0.0175965093316656	0.0281729513766027	0	0	84.95726495726495	343.2320001415874	RHOU
Positive Regulation Of Intracellular Protein Transport (GO:0090316)	1/126	0.0187819675844018	0.0281729513766027	0	0	79.488	315.953515922278	RHOU
Endocytosis (GO:0006897)	1/189	0.028084148529689	0.0374455313729188	0	0	52.68351063829788	188.21447440701544	RHOU
Regulation Of Intracellular Signal Transduction (GO:1902531)	1/297	0.0438936489206369	0.0526723787047643	0	0	33.27871621621622	104.02878903876676	RHOU

Table 2. GO Cellular Component 2023

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Cortical Granule (GO:0060473)	1/5	0.0007498314718703743	0.0029993258874814	0	0	2499.125	17982.858997820545	PADI6
Endosome Membrane (GO:0010008)	1/361	0.0531808446011125	0.0763000118067423	0	0	27.273611111111112	80.02232990571518	RHOU
Cytoplasmic Vesicle Membrane (GO:0030659)	1/389	0.0572250088550567	0.0763000118067423	0	0	25.269329896907216	72.28959580285303	RHOU
Bounding Membrane Of Organelle (GO:0098588)	1/819	0.1178932872819009	0.1178932872819009	0	0	11.723105134474327	25.06371049171824	RHOU

Table 3. GO Molecular Function 2023

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Hydrolase Activity, Acting On Carbon-Nitrogen (But Not Peptide) Bonds, In Linear Amidines (GO:0016813)	1/9	0.0013494318923464	0.0107954551387712	0	0	1249.3125	8255.5464452602	PADI6
GTP Binding (GO:0005525)	1/201	0.0298493008283308	0.053276626651987	0	0	49.4925	173.79755922762263	RHOU
Guanyl-Nucleotide Exchange Factor Activity (GO:0005085)	1/203	0.0301432849570313	0.053276626651987	0	0	48.99752475247525	171.5791941822581	RHOU
Guanyl Ribonucleotide Binding (GO:0032561)	1/226	0.0335198352152786	0.053276626651987	0	0	43.937777777777775	149.1959055604995	RHOU
GTPase Activity (GO:0003924)	1/265	0.0392273645668329	0.053276626651987	0	0	37.37310606060606	121.02834536731562	RHOU
Ribonucleoside Triphosphate Phosphatase Activity (GO:0017111)	1/270	0.0399574699889902	0.053276626651987	0	0	36.66914498141264	118.0724335197098	RHOU
GTPase Regulator Activity (GO:0030695)	1/424	0.0622639992823089	0.0697172961048257	0	0	23.1371158392435	64.23723782008254	RHOU
Purine Ribonucleoside Triphosphate Binding (GO:0035639)	1/476	0.0697172961048257	0.0697172961048257	0	0	20.549473684210525	54.72955384796592	RHOU

Table 4. HMDB Metabolites

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Guanosine triphosphate (HMDB01273)	1/460	0.067428196372244	0.0678576882736759	0	0	21.28322440087146	57.39430107698986	RHOU
Magnesium (HMDB00547)	1/463	0.0678576882736759	0.0678576882736759	0	0	21.14177489177489	56.87861734168601	RHOU

Table 5. miRTarBase 2017

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
mmu-miR-199a-5p	1/32	0.0047924977347936	0.0336249419150495	0	0	322.03225806451616	1719.8788256956173	RHOU
hsa-miR-126-3p	1/58	0.0086751325027492	0.0336249419150495	0	0	174.91228070175438	830.360139497581	RHOU
hsa-miR-452-5p	1/75	0.0112083139716831	0.0336249419150495	0	0	134.61486486486487	604.5687465206931	RHOU
hsa-miR-374b-5p	1/234	0.0346924480209845	0.0642745574039018	0	0	42.412017167381975	142.55668238733136	RHOU
mmu-miR-466l-5p	1/391	0.0575134362651423	0.0642745574039018	0	0	25.137179487179488	71.78516560137199	RHOU
mmu-miR-466d-5p	1/395	0.05809011459502	0.0642745574039018	0	0	24.87690355329949	70.79369144023838	RHOU
mmu-miR-466i-5p	1/395	0.05809011459502	0.0642745574039018	0	0	24.87690355329949	70.79369144023838	RHOU
mmu-miR-466k	1/395	0.05809011459502	0.0642745574039018	0	0	24.87690355329949	70.79369144023838	RHOU
mmu-miR-7b-5p	1/438	0.0642745574039018	0.0642745574039018	0	0	22.379862700228838	61.423578964976976	RHOU

Table 6. PPI Hub Proteins

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
PTK2	1/163	0.0242523103555676	0.0542353197780439	0	0	61.21913580246913	227.68886640394177	RHOU
PAK1	1/176	0.0261694857476556	0.0542353197780439	0	0	56.63428571428572	206.32783304510207	RHOU
NCK1	1/244	0.0361568798520293	0.0542353197780439	0	0	40.64609053497942	134.94046961461834	RHOU
PLCG1	1/244	0.0361568798520293	0.0542353197780439	0	0	40.64609053497942	134.94046961461834	RHOU
SRC	1/513	0.0749964858813518	0.0899957830576222	0	0	19.0283203125	49.2893249102792	RHOU
GRB2	1/767	0.110699237301363	0.110699237301363	0	0	12.552872062663186	27.62809726264245	RHOU

Table 7. Reactome Pathways 2024

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
RHOU GTPase Cycle	1/40	0.0059882314019791	0.0481908241377977	0	0	255.8717948717949	1309.541398623503	RHOU
M-decay Degradation of Maternal mRNAs by Maternally Stored Factors	1/46	0.0068844034482568	0.0481908241377977	0	0	221.6888888888889	1103.6774229731402	PADI6
Maternal to Zygotic Transition (MZT)	1/109	0.0162617282000212	0.0584737631112347	0	0	92.07870370370372	379.26673825723503	PADI6
Interleukin-4 and Interleukin-13 Signaling	1/112	0.0167067894603527	0.0584737631112347	0	0	89.57657657657657	366.541984609772	RHOU
Chromatin Modifying Enzymes	1/238	0.0352783986095116	0.0823162634221937	0	0	41.687763713080166	139.4240770668133	PADI6
Chromatin Organization	1/238	0.0352783986095116	0.0823162634221937	0	0	41.687763713080166	139.4240770668133	PADI6
RHO GTPase Cycle	1/450	0.0659956039535799	0.1159939195015217	0	0	21.76837416481069	59.17007947527356	RHOU
Signaling by Interleukins	1/452	0.0662822397151552	0.1159939195015217	0	0	21.66962305986696	58.807744555934576	RHOU
Signaling by Rho GTPases	1/672	0.0974554568631971	0.1395731862121099	0	0	14.400894187779434	33.53046394688685	RHOU
Signaling by Rho GTPases, Miro GTPases and RHOBTB3	1/688	0.0996951330086499	0.1395731862121099	0	0	14.053857350800582	32.403113451140854	RHOU

Table 8. Target Scan microRNA 2017

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
hsa-miR-126	1/153	0.0227758492784422	0.2706471536354705	0	0	65.27960526315789	246.8910278850476	RHOU
hsa-miR-4684-3p	2/1953	0.0267334566800765	0.2706471536354705	0	0	18.499231163505893	67.00124501584236	PADI6;RHOU
mmu-miR-450a	1/241	0.0357177059161049	0.2706471536354705	0	0	41.16041666666667	137.15098449199263	RHOU
hsa-miR-487b	1/262	0.0387891236974251	0.2706471536354705	0	0	37.80842911877394	122.8628530879148	RHOU
mmu-miR-652	1/315	0.0465117822885065	0.2706471536354705	0	0	31.34235668789809	96.15990539884224	RHOU
hsa-miR-4479	1/337	0.0497052271622002	0.2706471536354705	0	0	29.257440476190474	87.82045510023957	RHOU
hsa-miR-4665-3p	1/356	0.0524574576559142	0.2706471536354705	0	0	27.664788732394367	81.54895756877927	RHOU
hsa-miR-4304	1/487	0.0712888773429607	0.2706471536354705	0	0	20.07304526748971	53.0132128788392	RHOU
hsa-miR-3917	1/514	0.0751388888058125	0.2706471536354705	0	0	18.990253411306043	49.15469528186957	RHOU
mmu-miR-1963	1/525	0.0767043565059508	0.2706471536354705	0	0	18.581106870229007	47.71250625877883	RHOU

Table 9. RNA seq Automatic GEO Signatures Human Down

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Deep Gsk-126 Her2 Tumours GSE136300 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Circrna Gallbladder Compared Matched GSE100363 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Cyclooxygenase-2 Cox-2 Er? 5?-Reductase GSE80979 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Follicular Helper Effector Lymphocytes GSE58596 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Ppar? Prevents Infectivity Boosting GSE128121 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Protein Casp8Ap2 Improvement G0 GSE143808 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Moderate Dysplasia Mucosa Conversion GSE72627 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Arginine Integrating E2F Set GSE111960 3	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Gtf2I Williams Behavioural Rescuable GSE128840 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Cdk9 Degrader Multi-Targeted Cdk GSE89385 3	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU

Table 10. RNA seq Automatic GEO Signatures Human Up

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Transcriptional Cell-Derived Polarized Hepatocytes GSE123462 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Age-Induced Methylmalonic Acid Accumulation GSE127001 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Metallo-Endopeptidase Neprilysin White Preadipocytes GSE117270 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Ehmt1 Ehmt2 Fetal Hemoglobin GSE71421 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Polybrominated Diphenyl Ethers Pbdes GSE111203 4	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Directed Embryonic Corneal Cell-Like GSE81474 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Leader Recruitment Upstream Reading GSE81802 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Maintaining Iron Lysosomal Acidity GSE141507 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
U87Mg A3 Adenosine Mrs1220 GSE100146 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU
Strand-Oriented Culture 24H Presence GSE102305 1	1/250	0.0370348276752191	0.0370348276752191	0	0	39.65461847389558	130.69751905195764	RHOU

odds ratio=221.68), Maternal to Zygotic Transition (p=0.0162, adjusted p=0.0584, odds ratio=92.07), and Chromatin Modifying Enzymes/Organization (p=0.0352, adjusted p=0.0823, odds ratio=41.68).

Target Scan microRNA 2017

Table 8 indicates potential microRNA targeting of RHOU by hsa-miR-126 (p=0.0227, odds ratio=65.27), supporting the findings from miRTarBase. Both PADI6 and RHOU were targeted by hsa-miR-4684-3p (p=0.0267, odds ratio=18.49).

RNAseq Automatic GEO Signatures Human Down/Up

Tables 9 and 10 show differential expression of RHOU across various experimental conditions and disease states in GEO datasets, with consistent p-values of 0.0370 and odds ratios of 39.65 across multiple signatures, indicating the potential involvement of RHOU in diverse biological contexts beyond BCC.

DISCUSSION

Our comprehensive bioinformatic analysis of GWAS-identified genes associated with basal cell carcinoma has revealed significant functional implications for RHOU and PADI6 in disease pathogenesis.

RHOU is not yet an established clinical biomarker for any cancer, including BCC. However, several studies have reported RHOU overexpression in malignancies such as breast and colorectal cancers, with correlations to aggressive tumor behavior. Its expression could potentially serve as a prognostic indicator or part of a molecular signature when combined with other markers, especially if further validated in BCC-specific studies. With GWAS implicating RHOU in BCC susceptibility, future studies may explore its expression levels in patient samples as a non-invasive diagnostic or risk stratification tool.

The results highlight several molecular mechanisms through which these genes may influence BCC development and progression, providing novel insights into the underlying biology of this common skin malignancy.

RHOU, a Rho GTPase family member, is strongly linked to GTPase signaling, cytoskeletal organization, and endocytosis, aligning with its known role in cell migration and morphology. [29,30] Its association with Cdc42 signaling—a pathway involved in cancer invasion—suggests a role in BCC malignancy. RHOU also appears to influence mitochondrial function and energy metabolism, as indicated by its involvement in protein localization to mitochondria and interactions with energy-related metabolites (GTP, magnesium). Protein-protein interaction (PPI) analysis shows RHOU connects with key signaling molecules like PTK2 (FAK), PAK1, NCK1, and PLCG1, implicating it in focal adhesion, cytoskeleton remodeling, and cell invasion pathways. [31,32] Collectively, these findings position RHOU as a potential driver of BCC pathogenesis through its effects on cell signaling, metabolism, and motility. Our microRNA interaction analysis identified several microRNAs targeting RHOU, with particularly strong associations with miR-199a-5p, miR-126-3p, and miR-452-5p. [33]

These microRNAs have been previously implicated in cancer biology, with miR-199a-5p shown to regulate cell migration and invasion in various malignancies,[34] miR-126-3p known to modulate angiogenesis and metastasis,[35] and miR-452-5p involved in epithelial-mesenchymal transition.[36] The identification of these regulatory relationships provides insight into the post-transcriptional control of RHOU expression and suggests potential mechanisms through which dysregulation of these microRNAs could contribute to BCC pathogenesis.

The Reactome pathway analysis further confirmed the involvement of RHOU in GTPase cycle regulation and revealed an unexpected association with interleukin-4 and interleukin-13 signaling. These cytokines play roles in immune regulation and have been implicated in the tumor microenvironment of various cancers.[37] This finding suggests a potential link between RHOU and immune-related processes in BCC, which warrants further investigation given the emerging importance of immunotherapy in skin cancer treatment.

PADI6, while less extensively characterized in the context of cancer, showed significant associations with cellular components related to cortical granules and molecular functions involving hydrolase activity. The most striking findings for PADI6 came from the Reactome pathway analysis, which revealed strong associations with maternal mRNA degradation, maternal-to-zygotic transition,

and chromatin organization. PADI6 has been primarily studied in the context of early embryonic development, where it participates in epigenetic regulation and cytoplasmic lattice formation.[38] The significant enrichment in chromatin modifying enzymes suggests that PADI6 may influence BCC pathogenesis through epigenetic mechanisms, potentially affecting gene expression patterns critical for cell identity and differentiation.

The association of both RHOU and PADI6 with hsa-miR-4684-3p in the Target Scan analysis suggests a potential co-regulation of these genes, which could be relevant to their roles in BCC. While the functional significance of this microRNA in cancer remains to be elucidated, this finding points to a possible regulatory network involving both genes of interest.

RHOU shows variable expression across disease states, indicating it may impact multiple biological processes relevant to cancer. [39] Its involvement in GTPase signaling and cytoskeletal regulation suggests it could be a promising therapeutic target in BCC, especially as Rho GTPase inhibitors are being explored in other cancers. Additionally, microRNAs regulating RHOU may serve as useful biomarkers. [40] Meanwhile, PADI6 is linked to chromatin modification and epigenetic dysregulation, highlighting its potential role in BCC and the relevance of epigenetic therapies like HDAC and DNA methyltransferase inhibitors in treating such cases.

Limitations of our study: The analyses are based on computational approaches and statistical associations, which require experimental validation. The functional implications we have identified represent testable hypotheses rather than definitive mechanisms. Additionally, the analyses are constrained by the current state of knowledge represented in the databases used, which may contain biases or gaps.

CONCLUSION

Our comprehensive functional analysis of GWAS-identified genes offers important insights into the molecular underpinnings of basal cell carcinoma (BCC) and lays the groundwork for numerous future research directions. By elucidating the potential involvement of RHOU in GTPase signaling, cytoskeletal remodeling, and mitochondrial function, as well as the role of PADI6 in epigenetic regulation, this study enhances our understanding of BCC pathogenesis. These findings may ultimately contribute to the identification of novel therapeutic targets and support the development of more precise treatment strategies for this prevalent skin malignancy.

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Informed Consent Statement

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This research does not involve any clinical trials.

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Not Applicable.

Author contributions

Usha Adiga: Conceptualization, Methodology, Writing – Original Draft; Sampara Vasishta: Data Collection, Analysis, Writing – Review & Editing; Amulya Tunuguntla, Tulasi Govardhan: Visualization, Project Administration; Kasala Farzia: Funding Acquisition, Resources, Supervision.

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