# Bioinformatics Analysis of Gene Targets for Birt-Hogg-Dube Syndrome Associated with Renal Cell Cancer using NetworkAnalyst

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Abstract: CrRCC (chromophobe renal cell cancer) belongs to the group of non-clear cell cancer which accounts 4%-5% of RCC. Birt-Hogg-Dube Syndrome (BHDS), a subtype for crRCC, occurs due to the germline mutation of Folliculin (FLCN). Each disease has designated treatment and contrasting prognosis, but the histological features of this syndrome may overlap with the other subtypes of RCC which makes it difficult to differentiate and it has a limited amount of information available due to its uncommonness. This study aims to differentiate the pathway and genes involved in BHDS disease through NetworkAnalyst. The dataset was gathered from ArrayExpress and generated 395 significant DEGs in BHDS, which was then used to produce a pathway enrichment network and protein-protein interaction (PPI). Cytoskeletal protein binding correlating with hub genes KIT, RHOB, and UBC in BHDS indicates that this disease has a high risk for cell metastasis. This study gives a new promising therapeutic target for the said disease.

## 1 INTRODUCTION

Every year, there are approximately 338,000 new renal cell carcinoma releases in the world and about 30% of new renal cell carcinoma patients have metastases at the time of diagnosis (Li et al., 2021). Renal cell carcinoma (RCC) is a frequently diagnosed cancer with high prevalence (Y. Y. Chen et al., 2020). It is a heterogeneous tumor that derives from epithelial cells of the renal tubular, which represents a comprehensive 80% of all main RCC kidney tumors (Singh, 2021). Obesity, hypertension, and cigarette smoking are well-known risk factor for RCC although their impact may be different depending on the population. Renal cell carcinoma is more prone to male gender than females and a high incidence is generally seen from the sixth to eight decades of its existence that proves gender, race, and age affects the occurrence of RCC (Thompson et al., 2008). Genes that are typically involved in renal cell carcinomas such as VHL, MET, FLCN, SDH, TSC1, and TSC2 have an important role regarding with regulation of cellular metabolic processes which suggest a dysregulation of metabolic pathways involved in oxygen, energy, and/or nutrient sensing as a key feature of RCC carcinogenesis (Linehan, Srinivasan, & Schmidt, 2010).

RCC has many histological subtypes with different molecular drivers in which clear cell RCC is the most prevalent subtype, approximately for about 75%. The remaining subtypes include papillary renal cell cancer (pRCC), chromophobe renal cancer (crRCC), MiT family translocation, and other rare types (F. Chen et al., 2016). Most genomic alterations in RCC were well defined until the World Health Organization (WHO) in 2016 discovered classifications of tumors included subtypes which include Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC), von Hippel-Lindau disease (VHL), Birt-Hogg-Dube Syndrome (BHDS), and Hereditary Papillary renal carcinoma (HPRCC) (Moch, Cubilla, Humphrey, Reuter, & Ulbright, 2016).

Birt-Hogg-Dube Syndrome (BHDS) is a major autosomal dominantly inherited syndrome. BHDS is mostly involved with chromophobe renal cell carcinoma (crRCC), which is the third common subtype of RCC as it accounts 4%-5% of the incidence rate. This syndrome is associated with other benign or malignant tumors in other organs. Patients with BHDS deal with the RCC subtype chromophobe cell RCC, which is often considered as the counterpart of the benign oncocytoma, own hybrid forms (oncocytoma-chromophobe) (Murphy, Burns, Murtagh, Rooshenas,



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& Caskey, 2021). This hereditary syndrome is becoming more evident due to the advancement in pathological and molecular characterization and since there are many histological features are associated with distinct RCC hereditary, overlapping of their features is possible (Carlo et al., 2019).

It is important to identify patients at risk for hereditary RCC, as it may influence care (e.g. radical versus partial nephrectomy and surveillance type and schedule) and family members at risk could be offered specific screening to enable early detection. Each subtype is endowed with its unique risk factors, prognosis, prevalence, survival rate, responsiveness to diverse therapeutic agents, and clinical outcomes. Furthermore, the main treatment is surgery combined with chemotherapy and immunotherapy, but the therapeutic effect is limited (Fisher, Gore, & Larkin, 2013). Therefore, it is necessary to further study the pathogenesis of BHD syndrome to find possible early diagnostic markers and therapeutic targets.

The objectives of the study are identifying the main gene/s concern in differentiating the pathogenesis on different classifications of RCC specifically on BHDS disease and then integrating it on web-tool based mainly named NetworkAnalyst that will enable the user to construct a protein-protein interaction network that will aid classification of the pathophysiological pathways of this subtype of RCC.

This study focuses on the genes and pathways that are needed to differentiate the specific subtype of RCC therefore aimed at one organ, which is the kidney. As the NetworkAnalyst is used as the main-tool-based software, it also limits the resource of collected microarray data by choosing the ArrayExpress as the library resource for the dataset of BHDS (E-GEOD-21816). It is also noted that research on human tissues is used in conducting this study on gene expressed table.

# 2 METHODOLOGY

## 2.1 Data Collection

The gene expression dataset of BHDS [E-GEOD-21816 (GPL10175 Platform)] was manually searched and gathered from ArrayExpress database. The ArrayExpress (https://ebi.ac.uk/arrayexpress) is an open-source platform for the storage of genetic data. The E-GEOD-21816 dataset includes 6 normal kidney tissues and 6 Birt-Hogg-Dube syndromes associated with renal tumors patients. These microarray datasets (BHDS kidney tissues vs. normal kidney tissues and HLRCC kidney tissues vs normal kidney tissues) are inputted in text file (.txt) and are uploaded in NetworkAnalyst. Table 1 consists of the inclusions and exclusions criteria for the microarray dataset.

Table 1: Inclusions and Exclusions criteria for microarray datasets.

| Inclusion | Exclusion |
|---|---|
| Kidney tissues | No other organ tissues |
| Homo sapien organism | Non-human organism |
| BHDS | No other subtypes of disease |

## 2.2 Data Preprocessing, Quality Check, and Normalization

In uploading, the gene expression table, data should be specified according to their specific organism, data type, ID type, and gene-level summarization. Both datasets are specified as homo sapien, microarray data, Entrez ID, and mean, accordingly. After the datasets are successfully uploaded, a quality check and normalization of the data are done to enable to have more refined data analysis. Diagnostic plots such as box plots and density plots are included in both the quality check category and the normalization category. These diagnostic plots give different perspectives on the data. The distribution of gene expression values can be seen through these diagnostic plots and the results of different normalization methods on sample clustering can be visualized using PCA plots (G. Zhou et al., 2019). Box plots are applied to examine the normalization status. Log scale is applied if all data values are 20 while quantile normalization is used if all samples have identical distribution (Xia, Gill, & Hancock, 2015). The two datasets are filtered and further normalized to quantile normalization.

## 2.3 Identification of DEGs

NetworkAnalyst may also be used to distinguish DEGs between renal tumor tissue and normal kidney tissue samples. If one probe set does not contain the homologous gene, or if one gene has numerous probe sets, the data are removed (Li et al., 2021). Fold change of the genes present in BHDS tumor compared to normal kidney tissue were analyzed using the LIMMA package. The comparison of interest is set to its specific comparison (control vs. infected). To determine the genes that are significantly expressed on both datasets, the FDR adjusted p-values were kept to less than 0.05. Based on the fold change, genes were categorized into two classes, up-regulated genes (log2FC > 2) and down-

regulated genes (log2FC < -2), the cut-off statistic criteria were based in the study of L. Zhou et al. (L. Zhou, Li, Li, & Huang, 2020). Genes that were commonly up-regulated and down-regulated in both datasets were used to further analysis.

### 2.4 Pathway Enrichment Analysis

The pathway and process enrichment analysis were performed in all the common DEGs in both datasets. NetworkAnalyst allows users to perform functional enrichment analysis for highlighted nodes using different databases such as GO, KEGG, PANTHER, and Reactome pathway databases (Xia, Benner, & Hancock, 2014). KEGG, PANTHER, and Reactome are commonly used for biological information databases worldwide. The GO resource includes three aspects of biology which are biological process (BP), cellular component (CC), and molecular function (MF), and it is also commonly used in bioinformatics. The rule of significant is that P-value < 0.05 (Li et al., 2021).

### 2.5 Networking Mapping and Visual Analytics

This step deals with constructing PPI networks, heatmaps, volcano plots, and other visualization steps. The summary-level data (P values and fold changes) from the two datasets are extracted and integrated to identify genes that are significantly altered in expression, based on overall evidence (Xia et al., 2014). The significant genes of the datasets are presented in PPI networks and visualization analysis. In the PPI network, the number of nodes, edges, and seed proteins are summarized for each network (Xia et al., 2015). The clustering analysis of expression levels of hub genes is performed using interactive heatmaps or enrichment networks. The heatmap visualization tool shows detailed gene expression patterns underlying individual functions; while the enrichment network tool provides an overview of all enriched functions with similar ones connected by edges (G. Zhou et al., 2019) that uses different databases mentioned above.

## 3 RESULTS

### 3.1 Identification of DEGs

Following the preprocessing of the raw dataset, and then thoroughly running it through the LIMMA package, a total of 395 significant genes was identified in the dataset of E-GEOD-21816. With cut-off statistic criteria of p-value ≤ 0.05 and fold change (FC) ≥ 2 or FC < -2, each set has generated their own up and down regulated genes, for E-GEOD-21816 consists of 148 down-regulated genes and 247 up-regulated genes. The visualization of the resulted DEGs of BHDS dataset was done through volcano plots heatmaps (Figure 1). Based on the DEGs, heatmap analysis showed clear segregation of patients with BHDS from the control sample (Figure 1A and Figure 1B). The top up and down regulated DEGs ranked by fold change in BHDS is listed in Table 2.

### 3.2 Pathway Enrichment Analysis

It was concluded that while PPIs are reliable in discerning specific hub nodes that can describe the gene's centrality towards protein genes, it is only enclosed within a specific subnetwork, hence the usage of gene set enrichment analysis (GSEA). GSEA is primarily used as a visual data analysis within the NetworkAnalyst to produce gene count for the enriched KEGG and GO pathways. P-value was added to determine the probability of connection between the pathway and BHDS genes seen in Tables 3 & 4.

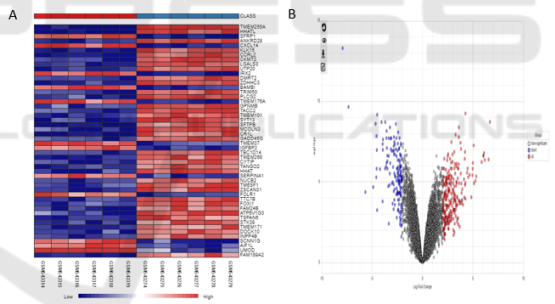


Figure 1: Hierarchical clustering heatmap of (A) BHDS and control sample. Volcano plot of DEGs between (B) BHDS sample and control sample. The red circles found in the volcano plots signify up-regulated genes while the blue circles are the down-regulated genes, and the white circles are non-significant genes. From the heatmap, the first six columns from the left are the normal kidney tissue and the last 6 columns are the tumor samples. The blue shade signifies low expressed genes while the red shade defines the high expressed genes.

### 3.3 Protein-Protein Interaction

Using both up and down regulated DEGs that were produced by the statistical analyzation of the provided sets, Hub nodes were identified through the string interactome database and therefore established the protein-protein interaction. As stated above, Table 5

shows PPI can be used to determine the specific subtype's centrality towards BHDS genes. Figure 3 contains the visual representation of PPI network from DEGs of BHDS.

# 4 DISCUSSION

The different types of renal cell carcinoma may pose some difficulty in differentiating histologically as many features of the subtypes overlap each other. And because they all have designated treatment and as well as contrasting prognosis, the utilization of gene-expression microarray analysis is therefore essential in the identification of molecular pathogenesis that will aid in distinguishing biomarkers that is important in clinical diagnosis, especially in diseases where there is a limited amount of information available due to the rarity of some disorders (Caliskan, Andac, & Arga, 2020).

With that in mind, NetworkAnalyst was chosen as the designated program that will generate gene expression profiles as an innovative move to further test the program if it is accurate enough to be used not only to detect biomarkers but also construct pathways specifically for BHDS.

As previously stated, renal cell carcinoma has a high prevalence rate (Fisher et al., 2013) and within the aforementioned subtype; chromophobe, though not as much predominantly known as clear cell RCC is shown more significant than its counterparts. And from the previously gathered studies, BHDS has been mentioned the most by papers by the papers amongst the tumors that are enclosed within the chromophobe subtype and was therefore selected to be analyzed thoroughly.

## 4.1 Birt-Hogg-Dube Syndrome (BHDS)

Birt-Hogg-Sube syndrome (BHDS), a subtype of chromophobe renal cell cancer (crRCC), is a hereditary condition characterized by skin fibrofolliculomas, pulmonary cysts, spontaneous pneumothoraces, and multiple RCCs (Nickerson et al., 2002). The germline mutation in the folliculin (FLCN) gene affects this disorder but its function remains unknown.

As DEGs from the dataset were used to produce the pathway enrichment network analysis using GSEA (Figure 2), therefore it conveys a much larger visualization in terms of connection of gene towards the disease. Referring to Tables 3 & 4 that include the KEGG and GO pathways enriched from the DEGs of BHDS.

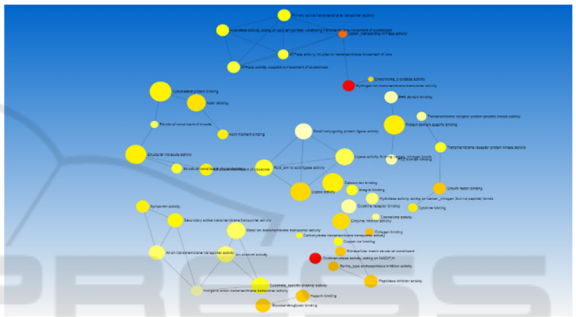

Figure 2: Visual representation of GO: MF pathway enriched in BHDS using GSEA network.

## 4.2 Pathway for BHDS

From reviewing the result in Table 4, it was interpreted that while the research of Moch et al. may say that the chromophobe form of RCC has a lowered risk for metastasis (Moch & Ohashi, 2021), but its subtype; specifically BHDS showed that cytoskeletal protein binding has the highest genome count with

Table 2: The top up and down regulated DEGs of BHDS ranked by log2FC.

| Up-regulated genes | | | Down-regulated genes | | |
|---|---|---|---|---|---|
| Gene Symbol | Log2Fc | P-value | Gene Symbol | Log2FC | P-value |
| Birt-Hogg-Dube Syndrome (BHDS) | | | | | |
| CXCL14 | 6.689 | 7.1641E-13 | TMEM255A | -7.9156 | 3.856E-18 |
| ALDOB | 6.558 | 2.346E-9 | HHATL | -7.3352 | 3.413E-14 |
| CALB1 | 6.177 | 2.567E-9 | DAPL1 | -5.6567 | 1.465E-6 |
| UMOD | 6.106 | 6.362E-10 | PVALB | -4.8824 | 5.800E-7 |
| NAT8 | 6.048 | 2.388E-7 | CKMT2 | -4.6471 | 1.508E-12 |
| PDZK1IP1 | 6.0416 | 1.1997E-9 | PDZK1IP1 | 6.0416 | 1.1997E-9 |
| ASS1 | 5.8721 | 3.8722E-9 | ASS1 | 5.8721 | 3.8722E-9 |
| PAH | 5.3302 | 6.6718E-8 | PAH | 5.3302 | 6.6718E-8 |
| BBOX1 | 5.2498 | 1.6913E-7 | BBOX1 | 5.2498 | 1.6913E-7 |
| PROM1 | 5.1054 | 2.7515E-7 | PROM1 | 5.1054 | 2.7515E-7 |

Table 3: Top KEGG pathways in the enrichment analysis of significant DEGs associated with BHDS.

| Birt-Hogg-Dube Syndrome (BHDS) | | |
|---|---|---|
| Pathway | Gene Count | P. value |
| Focal adhesion | 168/199 | 1.57E-4 |
| Fluid shear stress and atherosclerosis | 119/139 | 1.64E-4 |
| Cell adhesion molecules | 105/146 | 1.64E-4 |
| Leukocyte transendothelial migration | 89/112 | 1.68E-4 |
| TGF-beta signaling pathway | 71/92 | 1.71E-4 |
| Protein digestion and absorption | 70/90 | 1.72E-4 |
| Biosynthesis of amino acids | 61/75 | 1.75E-4 |
| Complement and coagulation cascades | 59/79 | 1.75E-4 |
| Glycolysis/Gluconeogenesis | 51/68 | 1.77E-4 |
| Oxidative phosphorylation | 106/133 | 2.55E-4 |

Table 4: Top GO (BP, MF, CC) terms in the enrichment analysis of significant DEGs associated with BHDS.

| PATHWAY | GENE COUNT | P-VALUE |
|---|---|---|
| Biological Process (BP) | | |
| Wound Healing | 483/610 | 1.4E-4 |
| Positive regulation of cell proliferation | 478/668 | 1.4E-4 |
| Regulation of anatomical structure morphogenesis | 478/605 | 1.4E-4 |
| Regulation of body fluid levels | 469/595 | 1.4E-4 |
| Vasculature development | 429/523 | 1.43E-4 |
| Response to biotic stimulus | 432/614 | 1.43E-4 |
| Response to other mechanism | 413/586 | 1.44E-4 |
| Negative regulation of development process | 404/563 | 1.44E-4 |
| Regulation of growth | 408/518 | 1.44E-4 |
| Negative regulation of cell proliferation | 409/526 | 1.44E-4 |
| Molecular Function (MF) | | |
| Cytoskeletal protein binding | 497/635 | 1.39E-4 |
| Calcium ion binding | 442/662 | 1.41E-4 |
| Structural molecule activity | 428/624 | 1.43E-4 |
| Actin binding | 278/356 | 1.51E-4 |
| Metal ion transmembrane transporter activity | 267/373 | 1.52E-4 |
| Enzyme inhibitor activity | 229/322 | 1.54E-4 |
| Substrate specific channel activity | 228/376 | 1.54E-4 |
| Anion transmembrane transporter activity | 165/229 | 1.60E-4 |
| Secondary active transmembrane activity | 146/192 | 1.61E-4 |
| Glycosaminoglycan binding | 136/172 | 1.63E-4 |
| Cellular Component (CC) | | |
| Cell surface | 351/488 | 1.47E-4 |
| Extracellular matrix | 313/424 | 1.48E-4 |
| Actin cytoskeleton | 292/366 | 1.5E-4 |
| Proteinaceous extracellular matrix | 262/362 | 1.51E-4 |
| Cell-cell junction | 232/292 | 1.54E-4 |
| Apical plasma membrane | 169/212 | 1.62E-4 |
| Extracellular matrix part | 139/178 | 1.64E-4 |
| External side of plasma membrane | 142/202 | 1.64E-4 |
| Apical junction complex | 96/117 | 1.7E-4 |
| Anchored to membrane | 97/146 | 1.7E-4 |

Table 5: The top 10 significant hub genes of BHDS according to their betweenness and their designated p-values.

| Hub Nodes | P-value | Betweenness |
|---|---|---|
| | BHDS | |
| KIT | 7.00E-9 | 102791.3 |
| RHOB | 2.74E-9 | 68625.94 |
| UBC | 0.062274 | 63768.85 |
| PLG | 0.003165 | 40525.61 |
| AGT | 4.70E-5 | 40265.16 |
| THBS1 | 4.09E-7 | 38286.66 |
| SRC | 0.36754 | 33653.63 |
| KNG1 | 2.34E-5 | 30817.65 |
| FHL2 | 7.28E-6 | 29003.64 |
| PRKAR2B | 8.04E-7 | 27625.94 |

497/635 and has significantly lowered p-value of 1.39E-4 which means that a lot of genes that is involved in cytoskeletal protein binding pathway is included with the progression of BHDS. This in turn, may potentially point out that unlike the previously constructed views of BHDS, it may possibly have a higher risk of metastasis. Figure 4 shows a schematic representation of the mechanism of cytoskeletal protein binding pathway enriched in BHDS.

### 4.2.1 Cytoskeletal Protein Binding

Cytoskeletal proteins contain different sub-families of proteins mainly which are Microtubules, Actin, and Intermediate Filaments (Pacheco & Gallo, 2016). The mechanism of these proteins is altered in cancer cells as they promote tumor growth by increasing the cells' migratory and invasive function alongside its ability to proliferate and the resistance to cellular

environmental stress such as: mitochondrial and oxidative stress (Allen et al., 2020). Mutations from these genes may result in metastasis and therefore and because of the high genome count and the significance of its p-value, this may very well allude to the possible metastatic characteristic of BHDS.

## 4.3 Hub Nodes for BHDS

In alignment with the statement above, three hub nodes were selected in terms of increasing betweenness. As this describes the gene's centrality towards other genes that are involved in the diseases' progression. These are: KIT (betweenness: 102791.3), RHOB (betweenness: 68625.94), and UBC (betweenness: 63768.85) in descending order.

### 4.3.1 c-KIT Gene Expression

C-KIT proto-oncogene is located on chromosome 4q and is considered to be part of class III of tyrosine kinase receptor (TKR) family. It is known to regulate several physiological functions such as: hematopoiesis, erythropoiesis, lymphopoiesis, megakaryopoiesis, gametopoiesis, and melanogenesis (Martinez-Anton, Gras, Bourdin, Dubreuil, & Chanez, 2019). All of these are essential to the biological process of human beings. Numerous research have suspected that this particular gene could be a potential biomarker to the chromophobe type of RCC as evidences show that it is found 77% to 100% in cases of this type of variety, and therefore is also a potentially targeted for therapeutic modalities (Yamazaki et al., 2003).

Using NetworkAnalyst, Figure 5 shown that C-KIT is connected to a gene called RAC1. RAC1 is considered as one of the key regulators for cellular motility and structure as the members of RAC family is considered to hold regulatory functions over cytoskeletal structures, mainly Actin (Tejada-Simon, 2015). As it primarily controls the mechanism behind the moderation of other signaling pathways that are involved in cell cycle regulation, cellular growth, formation of cell-cell adhesion, and contact inhibition, and these mediated activities are considered to be highly involved in progression of malignancy as it is included in angiogenesis, invasion, and metastasis which are dependent from the mutations from the genes assigned in it (Olson & Sahai, 2009).

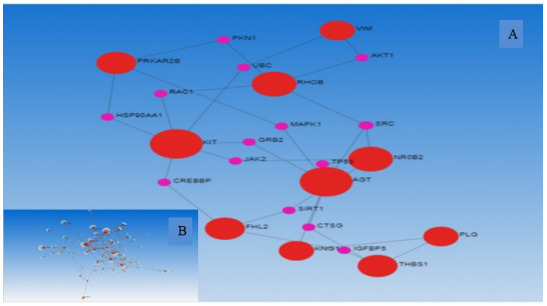


Figure 3: (A) The top 10 extracted hub nodes (Left to Right, Top to Bottom: PRKAR2B, KIT, FHL2, UBC, RHOB, KNG1, AGT, SRC, THBS1, PLG) The red-colored gene are seed genes, while purple-colored gene are: protein gene. (B) Along with the overall presentation of protein-protein interaction network for BHDS.

### 4.3.2 RHOB Gene Expression

RhoB is part of the Ras Homolog gene gamily or better known as Rho subgroup of GTPase which is included along with RhoA and RhoC. This family of genes is critical for analyzing regulation of cellular action and modulation of cytoskeleton-mediated motion and adhesion, as well as protein trafficking (Haga & Ridley, 2016).

Rho GTPases functions are directed by conversion of GDP-bound inactive states to GTP-bound active states. This activation is caused by three factors: Guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDI). The switches between active and inactive form are critical in regulating intracellular signaling pathways (Gampel & Mellor, 2002; Haga & Ridley, 2016).

Though the three subgroups of Rho GTPase share similar homology, they have different functions. Mainly RhoB is believed to have a putative tumor suppressor role, compared to the other two, which is claimed to have an oncogenic association (Ju & Gilkes, 2018). This particular function of RhoB serves in the signaling pathways including the EGFR, RAS, PI3K/AKT/mTOR, and MYC pathways (Gutierrez et al., 2019).

### 4.3.3 UBC Gene Expression

Ubiquitin C gene is described as a stress-inducible gene, upregulated upon different cell treatments as well as in other diseases (Radici, Bianchi, Crinelli, & Magnani, 2013). As it is one of the main hub nodes shown in the table above that was detected through the use of NetworkAnalyst, upon further inspection, WNK4 a subfamily of WNK protein is the one associated gene connected to UBC (Figure 5).

Interestingly, recent studies about the link between WNK4 and Rho GTPases have emerged; it shows that WNK4 can be isolated in a complex with Rho-GDI (Zhang et al., 2009) and while it is observed that the expression of WNK4 was found to be correlated with invasiveness as with metastatic tumors such as infiltrative gliomas or in other neural tumor cells the exact relationship between the genes is still largely undetermined (Hong et al., 2007). In addition, WNK4 is required for the activation of extracellular signal-regulated kinases and Mitogen-activated Protein/ERK Kinases (MEKK2/3) pathways. Alongside other reports, this suggests that WNK4 is involved in many factors that attribute to carcinogenesis and is an important role in tumor cell growth and remodeling of extracellular matrix for tumor invasion (Sie et al., 2020).

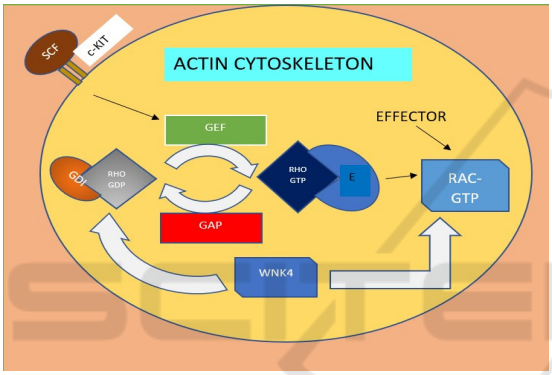


Figure 4: The mechanism of the pathway enriched in BHDS: Cytoskeletal protein binding. As C- KIT forms a heterodimer, SCF binds to it resulting to activation of RHO GTPase from inactive state. Then RHO GTPase will promote the activation of an effector such as RAC1 that regulates the Actin cytoskeleton. RAC1 can also be affected with the correlation of WNK4 with RHO GDI.

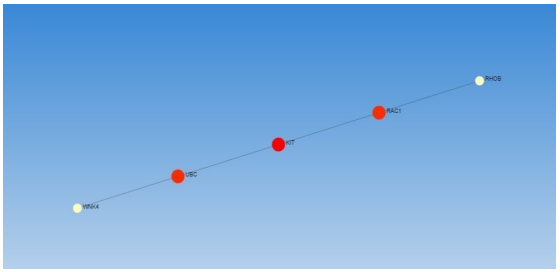

Figure 5: Protein-Protein Interaction of WNK 4 gene connecting to seed gene UBC and seed gene KIT to RAC1 gene.

And in that note, the connection between these genes and the given pathways may still up for further studies, they may support the suspicions that there are other characteristics traits of BHDS that have not been explored fully, and the presence of genes in the result above, bodes significance in terms of determinants for other biomarkers that may comprise this disease but also for alternative targeted treatments that may help patients in the future

## 5 CONCLUSION

The resulted hub genes from the PPI networks, which were ranked according to its betweenness, correspond with the high scored gene count and p-value enriched pathways. With the relation of the pathway and hub genes in the BHDS disease, it showed different pathophysiological features of this subtype of RCC. In this study, RAC1 and WNK4 genes were found to be connected to KIT, RHOB, and UBC respectively. These genes are known to highly affect the cell metastasis of patient with BHDS and play crucial role in Cytoskeletal protein binding, as these hub nodes control the regulation of cellular action and modulation of cytoskeletal structures.

In conclusion, this study gave significantly fresh insights for further examination on topics of diagnosis and the widening berth of therapeutic modalities for BHDS.

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