Genomic analyses identify significant genes and processes in adrenocortical carcinoma cells with overexpressed Ptch1

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Abstract

Adrenocortical carcinoma is a rare malignancy that mainly comes from family diseases. However, the mechanism and treatment of this cancer are still unclear today. Here, our objective is to determine the significant genes and signaling by analyzing the RNA-seq data from the Ptch1 overexpression cancer cells. The KEGG and GO analyses showed the Calcium signaling pathway and Pathogenic Escherichia coli infection were the key signaling pathways in Ptch1-overexpressed cancer cells. Moreover, we further identified the ten interactive molecules including ALB, STAT3, FOS, NRXN1, SNAP25, SYP, FYN, SPP1, THY1, GRIN2A. Our study may provide insights into the mechanism of Ptch1 regulating adrenocortical carcinoma.

Introduction

Adrenal tumors are common and benign in the human population, but adrenocortical carcinoma (ACC) is a rare endocrine malignancy¹. The surveillance database showed the incidence is approximately 0.72 per million cases per year in the USA¹. The reason for ACC is largely caused by the TP53 mutations, which account for 50%-80% of children with ACC². Thus, TP53 germline testing is recommended for patients with ACC³. Most ACC patients showed signs of hormone excess, and other patients indicate nonspecific symptoms caused by the tumor growth including the abdominal pain, early satiety or unrelated medical issues⁴. Recently, there are a couple of new modes of treatment such as receptors or enzymes⁵. Unfortunately, these medical trials do not meet the patients' needs due to various side effects.

Ptch1 was detected in various cancers such as lung cancers, colorectal cancers, and breast cancers⁶. There were significant associations between Ptch1 and Gli1 expression with large tumor size⁷. Moreover, the mediation of Smo activation by Ptch1 is changed in several cancers. Ptch1 inhibits Smo substoichiometrically to control the progression of cancers⁸. Ptch1 contains a GXXXD motif that is highly conserved in the resistance-nodulation-division family, and changes in GXXXD motif are essential for the activity of oncogene PTC-3⁹. Thus, Ptch1 may be a prognostic marker for high-risk cancer patients.

In our study, we analyzed the impact of Ptch1 on ACC by using the RNA-seq data. We figured out a number of DEGs and significant signaling pathways. We also performed the gene enrichment and created the protein-protein interaction (PPI) network to obtain the interacting signaling map and key molecules. These key genes and pathways in our study may provide novel insights for the treatment of ACC.

Methods

Data resources

Gene dataset GSE189424 was downloaded from the GEO database. The data was produced by the Illumina NextSeq 500 (Homo sapiens) (Functional Genomics Platform of Nice-Sophia-Antipolis, 660 route

des lucioles, Valbonne - Sophia-Antipolis, France). The analyzed dataset includes three controls and three Ptch1 overexpression H295R cell lines.

Data acquisition and processing

The data were organized and conducted by the R package as previously described^{10–13}. We used a classical t-test to identify DEGs with $P < 0.01$ and fold change ≥ 1.5 as being statistically significant.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO)

KEGG and GO analyses were conducted by the R package (ClusterProfiler) and Reactome. $P < 0.05$ was considered statistically significant.

Protein-protein interaction (PPI) networks

The Molecular Complex Detection (MCODE) was used to create the PPI networks. The significant modules were produced from constructed PPI networks and String networks. The biological processes analyses were performed by using Reactome (<https://reactome.org/>), and $P < 0.05$ was considered significant.

Results

Identification of DEGs in ACC cells after overexpression of Ptch1

To determine the effects of Ptch1 on ACC, we analyzed the RNA-seq data from the ACC cells with the overexpression of Ptch1. A total of 1160 genes were identified with the threshold of $P < 0.01$. The top up- and down-regulated genes were indicated by the heatmap and volcano plot (Figure 1). The top ten DEGs were listed in Table 1.

Enrichment analysis of DEGs after overexpression of Ptch1 in ACC cells

To further figure out the potential biological processes in ACC cells with the overexpression of Ptch1, we performed the KEGG and GO analyses (Figure 2). We identified the top ten KEGG signaling pathways, including “Calcium signaling pathway”, “Pathogenic Escherichia coli infection”, “Phospholipase D signaling pathway”, “Oxytocin signaling pathway”, “ECM–receptor interaction”, “Platelet activation”, “Complement and coagulation cascades”, “PPAR signaling pathway”, “Arrhythmogenic right ventricular cardiomyopathy”, and “Adipocytokine signaling pathway”. We identified the top ten BP items of GO enrichment, including “axon development”, “axonogenesis”, “cell junction assembly”, “regulation of nervous system development”, “synapse organization”, “axon guidance”, “neuron projection guidance”, “cell–cell adhesion via plasma–membrane adhesion molecules”, “regulation of synapse organization”, and “regulation of synapse structure or activity”. We identified the top ten CC items of GO enrichment, including “synaptic membrane”, “collagen–containing extracellular matrix”, “membrane raft”, “membrane

microdomain", "neuron to neuron synapse", "postsynaptic density", "asymmetric synapse", "postsynaptic membrane", "intrinsic component of synaptic membrane", and "intrinsic component of postsynaptic membrane". We also identified the top ten MF items of GO enrichment, including "DNA-binding transcription activator activity, RNA polymerase II-specific", "DNA-binding transcription activator activity", "peptide receptor activity", "cell adhesion mediator activity", "calcium-dependent protein binding", "cell-cell adhesion mediator activity", "scaffold protein binding", "extracellular matrix structural constituent conferring tensile strength", "ionotropic glutamate receptor activity", and "platelet-derived growth factor binding".

PPI network analysis

To determine the relationship among the DEGs, we create the PPI network by using 867 nodes and 2328 edges (Combined score > 0.2 as a cutoff, Cytoscape software). Table 2 showed the top ten genes with the highest scores. The top two significant clusters were indicated in Figure 3. We further analyzed the PPI and DEGs with Reactome map (Figure 4) and identified the top ten functional processes including "Response of EIF2AK1 (HRI) to heme deficiency", "Negative regulation of activity of TFAP2 (AP-2) family transcription factors", "Acyl chain remodelling of PG", "Ligand-receptor interactions", "Dissolution of Fibrin Clot", "Defective CHST3 causes SEDCJD", "Collagen degradation", "RUNX3 regulates RUNX1-mediated transcription", "CHL1 interactions", and "Acyl chain remodelling of PS" (Supplemental Table S1).

Discussion

Ptch1 is the most important Hh signaling regulator in cancers such as colorectal cancer, but the potential relationships between Ptch1 and patients' outcomes are still not clear¹⁴. Therefore, we herein use the data from the renal cancer cells with the overexpression of Ptch1 to assess the functions of Ptch1, thereby exploring the possible anti-cancer drugs.

By analyzing the KEGG and GO enrichment data, we found the "Calcium signaling pathway" and "Pathogenic Escherichia coli infection" were the key signaling pathways in Ptch1-overexpressed cancer cells. Yingying Hong et al found that Ptch1 siRNA decreases the SPARC levels, which affects the calcium metabolism¹⁵. Wu-Bo Li et al found that PTCH1 protein is a critical target for regulating the influenza virus infection. Moreover, PTCH1 was discovered to have association with decreased morbidity during the influenza infection¹⁶.

Besides the biological signaling, we also identified ten interacting molecules that were affected by the overexpression of Ptch1 in renal cancer cells. Sakae Konishi et al found that C-reactive protein/ALB ratio is a predictive marker for prognosis in cancer patients¹⁷. Keita Tamura et al also found the utility of the albumin can be considered as an objective prognostication tool to validate the metastatic cell carcinoma patients receiving second-line axitinib¹⁸. The activation of STAT3 can mediate multiple gene functions such as cell proliferation, differentiation, and apoptosis. Moreover, the inhibition of STAT3 was considered as an important therapy for cancer¹⁹. Circadian clocks regulate several downstream gene expressions through the transcriptional level to further mediate the cell functions such as proliferation,

differentiation, apoptosis, cell death, and metabolism^{20–31}. Zhenghui Tang et al found that STAT3 can inhibit the CRY2 expression through the CLOCK/BMAL1/P300 signaling³². Neil J Manimala et al found that FOS is a cellular proto-oncogene that increases the genes involved in proliferation and cancer formation³³. Takuma Yotsumoto et al found that NRXN1 is a novel drug target for cancers such as lung cancer³⁴. Qiongzheng Huang et al found that SNAP25 can inhibit the glioma progression through mediating synapse plasticity³⁵. Diana Rodica Tudorașcu et al found SYP is considered as a prognostic factor for colorectal cancer³⁶. Jörg Ellinger found that synaptophysin expression is associated with shorter cancer-specific survival of renal cancer patients³⁷. Daniel Elias et al discovered that FYN is a critical molecule in cancer pathogenesis³⁸. GPCR/RGS and their downstream signaling play vital roles in human physiological and pathological conditions, including aging, inflammation, metabolism homeostasis, and nervous system regulation^{39–50}. Interestingly, Rithwick Rajagopal et al found that Fyn is activated by GPCR stimulation and is responsible for further activating the Trk receptors on intracellular membranes⁵¹. Maj Rabjerg et al found SPP1 was identified as a promising novel prognostic marker for cancers⁵². THY1 contains critical roles in cancer, which regulates cancer cell proliferation, metastasis, and angiogenesis⁵³. X J Zhou et al found that the expression of GRIN2A showed different clinical significance between benign and malignant cancers⁵⁴.

In summary, our study found a strong relationship between Ptch1 and adrenocortical cancer. The Calcium signaling pathway and Pathogenic infection are the major affected processes in the Ptch1 regulated adrenocortical cancer. Based on these findings, our study provides valuable insights for the diagnosis and treatment of adrenocortical cancer.

Declarations

Author Contributions

Min Zhang, Hongmei Guo: Methodology and Writing. Hanming Gu, James Liu: Conceptualization, Writing-Reviewing and Editing.

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Declarations of interest

There is no conflict of interest to declare.

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Tables 1-2

Tables 1-2 are available in the Supplementary Files section.

Figures

Figure 1

Heatmap and volcano plot in ACC cells after overexpression of Ptch1

(A) Heatmap of significant DEGs. Significant DEGs ($P < 0.01$) were used to construct the heatmap. WT, wildtype; OE, Ptch1 overexpression.

(B) Volcano plot for DEGs in ACC cells after overexpression of Ptch1. The most significantly changed genes are highlighted by grey dots.

Figure 2

KEGG and GO analyses of DEGs in ACC cells after overexpression of Ptch1

(A) KEGG analysis (B) BP, Biological processes, (C) CC, Cellular components, (D) MF, Molecular functions.

Figure 3

The PPI network analyses of DEGs in ACC cells after overexpression of Ptch1

The cluster (A) and cluster (B) were constructed by MCODE.

Figure 4

Reactome map representation of the significant biological processes in ACC cells after overexpression of Ptch1 (yellow)

Supplementary Files

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