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Profiling of differentially expressed genes in cadmium-induced prostate carcinogenesis

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Abstract

The aim of the present study was to investigate the genetic signatures of cadmium-transformed prostate epithelial (CTPE) cells and to identify the potential molecular signaling involved in their malignant transformation. The dataset contained normal prostate epithelial (RWPE-1) and CTPE cells. To further examine the biological functions of the identified differentially expressed genes (DEGs), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome pathway enrichment analyses were performed. In total, 2,357 DEGs were identified, including 1,083 upregulated genes and 1,274 downregulated genes. GO, KEGG, and Reactome pathway enrichment analyses indicated that upregulated genes were significantly enriched in ECM–receptor, focal adhesion, TGF β signaling, and syndecan interactions, while downregulated genes were mainly involved in cell cycle regulation, arachidonic acid metabolism, oxidative phosphorylation, and folate biosynthesis ($p < 0.05$). The top upregulated (SATB1 ($p < 0.0001$), EYA2 ($p < 0.0001$) and KPNA7 ($p < 0.0027$)) and downregulated (PITX2 ($p < 0.0007$), PDLIM4 ($p < 0.0020$) and FABP5 ($p < 0.0007$)) genes were further validated via qRT-PCR analysis. In conclusion, the present study profiled DEGs in RWPE-1 and CTPE cells and identified gene pathways that may be associated with malignant transformation and tumor progression.

Keywords

Cadmium; prostate cancer; Differential gene expression; KEGG enrichment

1 Introduction

Cadmium (Cd) is a known human carcinogen that is known to be a risk factor for many malignancies (Kim *et al.*, 2018; Amadou *et al.*, 2019) including CaP (CaP) (Heath *et al.*, 1962; Armstrong and Kazantzis, 1985; Elinder *et al.*, 1985; Waalkes *et al.*, 1992; Jarup *et al.*, 1998; Waalkes *et al.*, 1999a; Waalkes *et al.*, 1999b; Pesch *et al.*, 2000; Achanzar *et al.*, 2001; Waalkes, 2003; Goyer *et al.*, 2004; Sorahan and Esmen, 2004; Adams *et al.*, 2012; Julin *et al.*, 2012). The International Agency for Research on Cancer (IARC) classified Cd as a

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Group 1 human carcinogen (Humans., 2018). Cd is a stable and persistent toxicant found in metal coatings, paints, and dyes, nickel–cadmium (NiCad) batteries, and jewelry (Lewis *et al.*, 1972; Aylett, 1979; Chmielnicka and Cherian, 1986; Friberg *et al.*, 1986; Agency for Toxic Substances and Disease Registry, 2012). Cigarette smoke is a significant source of exposure to Cd; the concentration of Cd in urine, blood, hair, and tissues has been correlated with smoking status (Hoffmann *et al.*, 2000; Paschal *et al.*, 2000; Adams and Newcomb, 2014). Cd has a biological half-life of > 20 years (Fox, 1983; Satarug *et al.*, 2003). Recent meta-analyses suggest that Cd levels in prostate tissues (standard mean difference [SMD] = 3.17, 95% confidence interval [CI] = 0.60–5.74, $p < 0.05$) and plasma (SMD = 4.07, 95 % CI = 2.01–6.13, $p < 0.05$) of CaP patients were significantly higher than those of healthy controls, suggesting that Cd is a risk factor for CaP (Zhang *et al.*, 2016b).

Prostate cancer (CaP) is a prominent type of cancer among men in the United States and worldwide (Siegel *et al.*, 2015). Clear dose-response relationships between Cd exposure and abnormal prostate-specific antigen (PSA) levels were found in volunteers working in Cd-polluted areas. Some case-control and epidemiological studies suggest an association between Cd exposure and CaP (Garcia Sanchez *et al.*, 1992; van der Gulden *et al.*, 1995; Vinceti *et al.*, 2007). Cd-exposed workers tested positive in the digital rectal exam, a non-invasive technique used to detect an inflamed prostate during clinical examinations (Zeng *et al.*, 2004; Zhang *et al.*, 2016b). Risk assessment studies have suggested that the likelihood of developing CaP in Cd-exposed cases was 1.10–2.76 times higher than in non-exposed subjects (van der Gulden *et al.*, 1995). Although some meta-analyses failed to correlate Cd exposure with the incidence of CaP (Chen *et al.*, 2016), the subjects were not substantially exposed to Cd; rather, they held diverse occupations in, for example, the hotel and catering, food, and retail industries. Interestingly, in the same review, the authors conducted a risk assessment of occupationally Cd-exposed individuals and found a positive correlation between CaP and Cd exposure by an odds ratio exceeding 2.0 (Chen *et al.*, 2016).

The primary and initial cellular mechanisms of metal exposure and toxicity are the production of free radicals that trigger oxidative stress, induce mutagenesis by DNA–metal interactions, and impair protein function by covalently modifying proteins or competing with metal binding sites (Joseph *et al.*, 2001; Jin *et al.*, 2003). Reactive oxygen species (ROS) production by metal exposure regulates many tightly controlled cellular processes (e.g., cell growth and proliferation), activate transcription factors and genes, and trigger processes such as inflammation and DNA repair (Shih *et al.*, 2004; Simmons *et al.*, 2009).

Identifying and understanding the molecular mechanisms of novel target genes for preventing disease progression has gained popularity in oncology studies (Ren *et al.*, 2016). Advancements in systems biology helps us to identify and predict the interactions within cells, which can be partly represented as gene networks. Analysis of these complex networks offers us meaningful insights into biological signaling pathways (Altay *et al.*, 2011). Cellular and biological processes are, at the genetic level, quite complex and become significantly altered when healthy cells adopt a malignant phenotype (Califano *et al.*, 2011; Emmert-Streib *et al.*, 2012). A single gene can possess multiple functions, including the regulation of expression of different genes, which may contribute to disease progression. Disease onset is

largely attributed to this complex interplay between genes and molecular processes (Menche *et al.*, 2015).

In this study, differential gene network analysis was performed to identify potential differences between normal prostate (RWPE-1) cells and cadmium-transformed prostate epithelial (CTPE) cells. Additionally, functional annotation and network analyses were performed to identify differential gene expression (i.e., DEGs). By analyzing the biological functions and networks of cadmium-mediated malignant transformation, the present study may help gain a better understanding of malignant transformation at the molecular level and explore the potential candidate biomarkers for diagnosis, prognosis, and treatment.

2 Materials and methods

2.1 RNA isolation, cDNA library construction, and DNA sequencing

RWPE-1 and CTPE cells were subjected to RNA isolation using TRIzol® (Invitrogen; Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. RNA concentration and sample purity were then assessed using a NanoPhotometer® spectrophotometer (Implen, Westlake Village, CA).

A cDNA library was then constructed using Novogene Bioinformatics Technologies (Beijing, China) and a NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA) according to the manufacturer's instructions. Briefly, mRNA were purified from RNA samples using poly-T oligo-attached magnetic beads and fragmented to ~200 bp using divalent cations under elevated temperature then reversely transcribed into cDNA using random hexamer primers and M-MuLV Reverse Transcriptase. The second strand cDNA was synthesized using RNase H, dNTP, and DNA polymerase I. Subsequently, polymerase chain reaction (PCR) was performed using Phusion High-Fidelity DNA Polymerase, Universal PCR primers, and Index (X) Primer for double-stranded cDNA amplification. PCR products were then purified using the AMPure XP system, the library quality was assessed using the Agilent Bioanalyzer 2100 system, and the cDNA library was sequenced using an Illumina HiSeq 2000/2500 platform and 100 bp/50 bp single-end reads were generated.

2.2 Data analysis

Downstream analysis was performed using a combination of programs including STAR, HTseq, Cufflink, and our wrapped scripts. Alignments were parsed using TopHat and differential expressions were determined through DESeq2/edgeR. GO and KEGG enrichment were implemented by the clusterProfiler.

2.3 Clustering

To identify the correlations between observed differences, we clustered different samples using expression FPKM values to see the correlation using a hierarchical clustering distance method with the function of heatmap, SOM (self-organization mapping) and kmeans using silhouette coefficient to adapt the optimal classification with default parameter in R.

2.4 GO, KEGG and Reactome enrichment analysis of DEGs

Gene Ontology (GO) enrichment analysis of DEGs was implemented by the clusterProfiler R package, in which gene length bias was corrected. GO terms with corrected $p < 0.05$ were considered significantly enriched by DEGs. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a resource database for understanding the high-level functions and utilities of a given biological system such as the cell, the organism, or the ecosystem at a molecular level, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies (<http://www.genome.jp/kegg/>). Reactome (<http://www.reactome.org>) databases was also used to perform signaling pathway enrichment analysis with $p < 0.05$ as a cut-off criterion. We used cluster Profiler R to test the statistical enrichment of DEGs in KEGG and Reactome pathways.

2.5 Differential expression analysis

2.5.1 DESeq2 with biological replicates—Differential expression analysis between two conditions/groups (two biological replicates per condition) was performed using the DESeq2 R package (2_1.6.3). DESeq2 provides statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting p values were adjusted using the Benjamini–Hochberg approach for controlling the False Discovery Rate (FDR). Genes with an adjusted $p < 0.05$ found by DESeq2 were considered differentially expressed.

2.5.2 edgeR without biological replicates—Prior to differential gene expression analysis, for each sequenced library, the read counts were adjusted with edgeR through one scaling normalized factor. Differential expression analysis of two conditions was performed using edgeR (3.16.5). The p values were adjusted using the Benjamini–Hochberg method. A corrected p value of 0.05 and absolute fold change of 1 were set as the threshold for statistically significant differential expression. The Venn diagrams were prepared using the function VennDiagram in R based on the gene list for each group.

2.6 qRT-PCR Analysis

Total RNA was isolated from cultured cells using TRIzol (Invitrogen). c-DNA was synthesized with a reverse transcription kit (applied biosystems) using SYBR Green qPCR superMix (Qiagen). The Primers used were: SATB1 (forward, 5′-TAGGTGACCAGGCAAAATGG-3′; reverse, 5′-CATGTGGGTTCTGCAGCTAA-3′), EYA2 (forward, 5′-ACGCTGCTGTGTGGACTCT-3′; reverse, 5′-AGAGCTGGGACACTCTCAGG-3′), WSCD1 (forward, 5′-GCTCCGTCTCTTGGTGAAC-3′; reverse, 5′-TCCATTCCAGGGCTCTACC-3′), KPNA7 (forward, 5′-CCAGCTCGTCCACTCTGG-3′; reverse, 5′-CTTCTCAGACCGTTTCTCTGC-3′), PITX2 (forward, 5′-ACCATCCCCAGCCGTTAG-3′; reverse, 5′-CCCTGCTGGCTTTTATCTTTC-3′), PDLIM4 (forward, 5′-TTTACCCTGTCCCTCTAATAAA-3′; reverse, 5′-CAGGCCAGGTGACAGGTT-3′), FABP5 (forward, 5′-GAGTGGGATGGGAAGGAAAG-3′; reverse, 5′-GATCCGAGTACAGGTGACATTG-3′), ELF3 (forward, 5′-CTGCAATGGCTTGGGTTC-3′; reverse, 5′-TCCAGAGCTCTTTGTAGCCAAT-3′).

Actin (forward, 5' - CTCCTCCACCTTTGACGCTG-3'; reverse, 5' - CATAACCAGGAAATGAGCTTGACAA-3') was used as an internal control

3 Results

3.1 Identification of DEGs in Cd-induced transformation

To investigate the molecular targets and mechanisms involved in Cd-induced transformation, gene expression analysis of Cd-exposed cells was carried out using RNA-Seq. We obtained 2,357 genes that were differentially expressed during Cd-induced transformation, of which 1,083 were upregulated and 1,274 were downregulated (the 10 most highly upregulated and downregulated genes are presented in Tables 1 and 2). Specifically, expression levels of PPAPDC1A, WSCD1, PDE10A, and EYA2 were the most highly upregulated in CTPE cells, whereas expression levels of H19, ELF3, PDLIM4, IFIT1, and UGT8 were the most highly downregulated. These DEGs were then clustered together in Figure 1A, where upregulated genes are coded as red and downregulated genes are coded as blue. The results confirmed that Cd-exposed transformed cells had differentially expressed genes relative to the prostate epithelial cells. A volcano map (Figure 1B) with $\log_2$ (fold change) as the abscissa and $-\log_{10}$ (padj) as the ordinate was used to demonstrate the overall distribution of DEGs among CTPE and RWPE-1 cells. In this volcano map, upregulated genes are presented as red dots and downregulated genes are presented as green dots. Blue dots represent unchanged genes. Then, we used Venn diagram software to identify the commonly DEGs in CTPE and RWPE-1 datasets. Results showed that a total of 11,365 commonly DEGs were detected. However, 746 DEGs were exclusively expressed in CTPE cells and 1564 DEGs were expressed in RWPE-1 cells (Figure 1C). The upregulated genes which are mainly involved in cell proliferation and focal adhesion; SATB1 and EYA2 expression in CTPE cells were increased by 7 and 20 fold respectively compared to RWPE-1 cells by qRT-PCR (Figure 1D). The downregulated genes such as PITX2, PDLIM4, FABP5 were involved in apoptotic stimuli and disrupting the cell cycle of the cells PITX2 ($p < 0.0007$), PDLIM4 ($p < 0.002$) and FABP5 ($p < 0.0007$) are significantly downregulated in CTPE cells compared to RWPE-1 cells (Figure 1E).

3.2 Functional enrichment of DEGs via GO and KEGG

DAVID analysis was performed to predict the potential functions and mechanisms of Cd-induced transformation by mapping the upregulated and downregulated genes using GO terms. The top GO terms of the BP, CC, and MF categories enriched by the upregulated and downregulated DEGs were identified (Figures 2 and 3). The results demonstrated that downregulated genes were mainly involved in chromosome segregation and mitotic nuclear division in the BP category (Figure 2A); in condensed chromosome, kinetochore and spindle formation in the CC category (Figure 2B); and in histone kinase, microtubule, and oxidoreductase activity in the MF category (Figure 2C). Upregulated genes were predominantly associated with extracellular structure and matrix organization in the BP category; were mainly intracellular (i.e., endoplasmic reticulum lumen, postsynaptic specialization, postsynaptic density, and focal adhesion) in the CC category; and were largely involved in surface binding (i.e., growth factor binding, glutamate receptor binding, misfolded protein binding, and receptor ligand activity) in the MF category (Figure 3C).

3.3 KEGG pathway analysis of DEGs

The results for the KEGG pathway enrichment are shown in Figure 4 and indicate that upregulated genes were significantly enriched in ECM–receptor interactions, focal adhesion, amoebiasis, TGF β signaling, cancer signaling, small cell lung cancer, and basal cell carcinoma (Figure 4B). Downregulated genes were enriched in processes of cell cycle regulation, oocyte meiosis, arachidonic acid metabolism, oxidative phosphorylation, Parkinson's disease progression, and folate biosynthesis (Figure 4C). Upregulation of ECM-related and TGF β signaling pathways renders the CTPE cells increasingly proliferative and invasive, thereby allowing them to form tumors.

3.4 Reactome pathway analysis of DEGs

In Figure 5, reactome-enriched pathways are described and indicate that upregulated genes were significantly enriched in ECM organization, syndecan and laminin interactions, and ECM proteoglycans (Figure 5B). Downregulated genes were enriched in interferon (IFN)- α/β signaling, cell cycle check points, Rho kinase signaling, and polo-like kinase-mediated events (Figure 5C). IFN- α/β and Rho kinase signaling are largely responsible for increased stress and inflammation in the cells, ultimately leading to tumorigenesis. This phenomenon correlates with the increased cellular proliferation and tumor-forming capacity in CTPE cells.

4 Discussion

Earlier we reported that Cd exposure manifests into a cancerous phenotype and transforms normal prostate epithelial cells (e.g., RWPE-1) to a malignant phenotype (e.g., CTPE). In the current study, we performed high-throughput RNA Seq to screen genes and pathways responsible for malignant transformation. We obtained 2,357 DEGs (1,083 upregulated; 1,274 downregulated) and observed that RWPE-1 and CTPE cells display distinct gene ontology, particularly genes associated with genitourinary carcinogenesis (i.e., PDE10A; 7 fold) and cancer cell survival (i.e., EYA2; 20 fold) and proliferation (i.e., KPNA7; 4 fold) are significantly upregulated.

PPAPDC1A is highly expressed in urothelial cancers and its involvement in rapid cell proliferation and tumorigenesis has been well noted (Zhang *et al.*, 2017). Higher PDE10A expression contributes to carcinogenesis, including CaP (de Alexandre *et al.*, 2015; Barone *et al.*, 2017; Zhu *et al.*, 2017). EYA2 expression is crucial for cell progression and tumor formation and is reported to be involved in multiple cancers (Wu *et al.*, 2013; Li *et al.*, 2017; Ono *et al.*, 2017). ELF3 expression was significantly lower in CaP, compared to other cancer types. ELF3 mRNA expression is lower in primary tumours compared to normal breast epithelium, and further reduced in distant metastases (Merino *et al.*, 2016). Shatnawi *et al.* reported a putative tumour suppressive function for ELF3 in CaP (Shatnawi *et al.*, 2014). PDLIM4 has been reported to act as a tumor suppressor in CaP. Overexpression of PDLIM4 significantly reduced the cell growth and colonogenicity in LNCaP, DU145 and PC3 cells (Vanaja *et al.*, 2006). Donkena *et al.* has reported that repression of PDLIM4 by hypermethylation is regarded as potential biomarker for CaP (Vanaja *et al.*, 2009). IFIT1 plays a key role in growth suppression and apoptosis promotion in cancer cells. High IFIT1

expression predicts improved clinical outcome in newly diagnosed glioblastoma (Zhang *et al.*, 2016a) .

Mapping of all DEGs to transcriptome background in KEGG pathway enrichment analysis revealed that 163 DEGs with pathway annotations corresponded to 21 pathways related to oncogenesis that were significantly upregulated and 196 DEGs with pathway annotations belonged to 4 pathways that were significantly downregulated, including that of the cell cycle. An overwhelming majority of upregulated genes were significantly enriched in ECM–receptor interactions, focal adhesion, TGF β and oncogenic signaling pathways, and basal cell carcinoma.

ECM comprises a complex network of molecules that are tightly regulated in order to maintain organ homeostasis. It is commonly deregulated in disease conditions and affects cancer progression by directly influencing cellular transformation and metastasis in CaP (Stewart *et al.*, 2004; Lu *et al.*, 2012). Modification of ECM proteins is associated with altered tumor histopathology (Provenzano *et al.*, 2008; Levental *et al.*, 2009) and with increasingly aggressive and invasive tumors in CaP (Penet *et al.*, 2017). ECM proteins were shown to be highly metastatic (Naba *et al.*, 2014) and transformation by oncogenes such as Ras, which stimulates Erk signaling to promote anchorage independence for growth and survival, simultaneously induces expression of several ECM proteins in CaP (Pattabiraman and Rao, 2010; Lang *et al.*, 2017). Pancreatic cancer patients with altered ECM protein pools display significant stromal desmoplasia followed by increased tumor progression and poor disease outcome (Pandol *et al.*, 2009). Similarly, expression of matrix remodeling genes such as MMPs and collagen cross-linkers is predictive of a poor prognosis for breast cancer patients. In our study, significant upregulation of integrin and growth factor-dependent signaling indicates that ECM is actively engaged and its organization is altered in CTPE cells.

Focal adhesion (FA) proteins are crucial complexes that form connections between the ECM and the cell's cytoskeleton with the help of integrins. Integrin-mediated adhesion of FA proteins to the ECM induces Src and Erk signaling and tumor suppressor levels of cell cycle inhibitors such as p27 remain low, promoting cell proliferation (Aguirre-Ghiso *et al.*, 2003; Pontier and Muller, 2008). Activation of β 1 integrin downregulates outside-in signaling of β 1 integrin, leading to the reduction of FA and fibronectin expression which, in turn, leads to cell adhesion and motility (Lee *et al.*, 2011) in human CaP cells. Genes associated with FA were found to be significantly upregulated in CTPE cells. A mechanistic link between cell–ECM contact and signaling for cell survival, proliferation, and differentiation is well established in various cancers (Legate and Fassler, 2009; Vehlow and Cordes, 2013). Together, the upregulation of genes associated with ECM organization, integrin proteins, and FAs in CTPE cells provide evidence and a mechanistic link for Cd-induced prostate carcinogenesis.

A plethora of research has been dedicated to determining how cellular components of the ECM niche initiate and promote cancer development (Bhowmick *et al.*, 2004). However, recent progress has also underlined the importance of other non-cellular cancer hallmarks such as NF- κ B, interleukins, and cytokines that are deregulated in response to metal-

induced chronic inflammation (Nguyen *et al.*, 2014). Disruption of the tumor microenvironment and deregulation of immune responses constitute the cellular events that contribute to prostate carcinogenesis in the occurrence of chronic inflammation. Reflecting a similar pattern, the current dataset also revealed several DEGs that annotated with growth factors, cytokines, chemokines, and components of ECM relevant to growth regulation in vascular repair, hypoxia, angiogenesis, and immune cell function, reflecting the sensitivity of these genes towards wound healing, inflammation, and vascular biology in RWPE-1 and CTPE cells.

In our study, we found 20% of genes were not intersecting in the Venn diagram; specifically, CTPE cells had higher proportions of genes relevant to inflammatory pathways such as IL1RL1 and adrenoceptor_alpha_1D (ADRA1D), which are involved in Ras activation through Gs- α and cAMP-mediated signaling (Newton and Dixit, 2012). Ras activation leads to inflammatory responses in various types of cancers (Ji *et al.*, 2006) that generates a progressive phenotype characterized by cellular atypia, adenoma, and, ultimately, adenocarcinoma (Vojtek and Der, 1998). In separate studies, our team has demonstrated that ROS-generated ER stress is the core signaling pathway activated during the transformation of RWPE to CTPE cells. Additionally, upregulation of NF- κ B and interleukins allow CTPE cells to proliferate in Cd-induced prostate carcinogenesis (Kolluru *et al.*, 2017). The current data from functional enrichment of DEGs indicate that these pathways are indeed activated in CTPE cells. Moreover, other signaling networks, such as integrin and FA proteins, together contribute to the changing landscape of oncogenes in malignant phenotypes.

The current study underlines several hallmarks of cancer and provides a substantial view of the transformative steps and determinants of malignant tumor progression in the event of chronic metal toxicity. Beyond that, it also illustrates a plethora of cellular and non-cellular targets. Upstream localization of the receptors within FAs make them an ideal starting point for therapeutic intervention. However, strong redundancy and complexity of downstream signaling in data from this study and crosstalk between these pathways are likely to challenge the efficacy of a particular molecular therapy. Nevertheless, the current data set can lead to identification of potential targets that require validation in various *in vitro* and *in vivo* models.

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Highlights

1. Genes that are most highly upregulated in Cd-exposed cells were involved in aberrant cell proliferation and tumorigenesis
2. The TGF β , RAS, interferon, and ECM-related signaling pathways were significantly active in CTPE cells
3. The upregulated and downregulated genes were validated using real time PCR and their expression correlates with DEG enrichment analysis.

Figure 1(A).

Hierarchical cluster of differentially expressed genes. Hierarchical cluster analysis was conducted for differential expression of genes using a fold change < 0.5 or > 2 and $p < 0.05$. The relative levels of DEGs are depicted to the color scale. Blue represents low levels, white represents medium levels, and red represents high levels. Samples represented are normal RWPE-1 and CTPE cells. (B) Volcano plot and Real time-PCR analysis. The detected genes are presented in the volcano plot using $\log_2$ (fold change) as x-axis and $-\log_{10}$ (padj) as y-axis. The upregulated genes in CTPE cells relative to RWPE-1 cells are presented in red, whereas downregulated genes are presented in green. Genes without significant diversity are presented in blue. (D & E) qRT-PCR analysis of top upregulated and downregulated genes are represented as fold between RWPE-1 and CTPE cells.

Figure 2. Gene Ontology (GO) terms of the top 20 downregulated enriched genes.

The GO enrichment analysis grouped these DEGs into functional groups, which are presented as (A) Biological Process (BP), (B) Cellular Component (CC), and (C) Molecular Function (MF).

Figure 3. GO terms of the top 20 upregulated enriched genes.

The GO enrichment analysis grouped these DEGs into functional groups, which are presented as (A) Biological Process (BP), (B) Cellular Component (CC), and (C) Molecular Function (MF).

Figure 4. KEGG pathway analysis.

KEGG pathway enrichment analysis was performed by Fisher exact test. Significantly enriched KEGG pathways ($p < 0.05$) are presented. For each KEGG pathway, the bar indicates the fold-enrichment of the pathway.

Figure 5. Reactome pathway analysis.

The Reactome pathway enrichment analysis of DEGs. 20 statistically significant pathways are listed including upregulated and downregulated pathways.

Table 1.

List of top 10 up-regulated genes in CTPE cells

Gene Symbol	Log2(fold change)	p-value
PPAPDC1A	8.6038	5.48E-27
WSCD1	8.5977	6.15E-32
PDE10A	7.8259	9.21E-22
EYA2	7.4045	1.93E-33
SATB1	7.1287	2.24E-17
ADRA1D	7.1166	2.59E-17
IGF2	7.0278	2.26E-39
C5AR2	6.7579	3.16E-15
KPNA7	6.5921	2.38E-14
VSTM2L	6.2572	3.56E-28

Top Up-regulated Genes

Table 2.

List of top 10 down-regulated genes in CTPE cells

Gene Symbol	Log2(fold change)	p-value
H19	−10.177	3.86E-54
ELF3	−9.3526	2.70E-38
PDLIM4	−9.0385	5.26E-36
IFIT1	−8.6104	2.96E-39
UGT8	−8.5524	7.19E-27
FABP5	−8.0308	5.16E-35
PITX2	−7.6907	4.59E-21
SPARC	−7.6065	8.44E-36
VANGL2	−7.5833	1.25E-35
FOXN1	−7.533	2.52E-25

Top down-regulated Genes