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Genome-wide Profiling of TRACK Kidneys Shows Similarity to the Human ccRCC Transcriptome

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Abstract

Renal cell carcinoma (RCC) is the most common cancer arising from the kidney in adults, with clear cell carcinoma (ccRCC) representing the majority of all RCCs. Expression of a human HIF1a triple mutant (P402A, P564A and N803A) construct in the proximal tubule cells of C57BL/6 mice (TRAnsgenic model of Cancer of the Kidney (TRACK) (1)) mimics the histological changes found in early stage human ccRCC. To better understand the genomic landscape, a high throughput sequence analysis was performed with cDNA libraries (RNAseq) derived from TRACK transgenic positive (TG+) kidney cortex along with human ccRCC transcripts from the Oncomine and TCGA databases. Importantly, the expression profiles of TRACK TG+ kidneys show significant similarities with those observed in human ccRCC, including increased expression of genes involved in glycolysis and the tricarboxylic acid cycle (TCA cycle). Some of the transcripts overexpressed in both the TRACK mouse model and human ccRCC include: ANKRD37, CA9, EGLN3, HK2, NDUFA4L2, and SLC16A3. These data suggest that constitutive activation of HIF1a in kidney proximal tubule cells transcriptionally re-programs the regulation of metabolic pathways in the kidney and that HIF1a is a major contributor to the altered metabolism observed in human ccRCC.

Implications—TRACK (GGT-HIF1αM3) kidney mRNA profiles show similarities to human ccRCC transcriptome and phenotypes associated with the Warburg effect.

Keywords

clear cell renal cell carcinoma; kidney cancer; RNAseq; HIF1α

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Introduction

Renal cell carcinoma (RCC) is the most common primary cancer arising from the kidney in adults, with clear cell carcinoma (ccRCC) representing ∼75% of all RCCs (2, 3). Histologically, ccRCC cells are characterized by a transparent cytoplasm caused by deposition of glycogen, phospholipids, and neutral lipids, including cholesterol esters (4, 5). This phenotype suggests that there are metabolic changes in ccRCC cells resulting in abnormal deposition of glycogen and lipids. Seven genes commonly mutated in human kidney cancer, including VHL (NCBI Gene ID: 7428), MET (4233), FLCN (201163), TSC1 (7248), TSC2 (7249), FH (2271), and SDH (6390, 6391 and 6392), have been identified to date (6). Interestingly, all seven genes are involved in the regulation of metabolic pathways (6). These data support the theory that kidney cancer is a metabolic disease (6, 7).

Loss of expression or mutation of the von Hippel-Lindau (VHL) tumor suppressor gene is found in hereditary and most sporadic ccRCCs (2, 8). This suggests an etiological role for VHL gene loss in renal carcinogenesis. However, the exact pathway by which loss of VHL leads to ccRCC has not been definitively elucidated. The best studied and likely most important effect of VHL loss is the resulting increase in expression of the alpha subunits of hypoxia inducible factors 1 (HIF1α) and 2 (HIF2α) (9-11). Increased expression of these two transcription factors has been proposed as a key step in ccRCC carcinogenesis (9, 10) [for review, see (12)]. We previously generated the murine TRAnsgenic model of Cancer of the Kidney (TRACK) that expresses a triple mutant (P402A, P564A and N803A) human HIF1α construct in murine proximal tubule cells (PTCs) and showed that this model mimics the histological alterations found in early stage human ccRCC (1). The cellular histologies displayed in TRACK mice are also similar to those observed in the kidneys of individuals with VHL disease, including areas of distorted tubular structure, cells with clear cytoplasm and increased glycogen and lipid deposition, multiple renal cysts, and areas of multifocal ccRCC (1).

To delineate the gene expression pattern in TRACK TG+ kidneys, we sequenced the entire transcriptome of the TRACK TG+ kidney cortex by high throughput sequencing of cDNA libraries (RNAseq) and compared it with both the gene expression profile in WT/TG- kidneys and the gene expression profile observed in sporadic human ccRCC using the Oncomine database (Compendia Bioscience, Ann Arbor, MI) and the TCGA database. We report that the pattern of gene expression in TRACK TG+ kidneys is similar to that of human ccRCC, including expression of transcripts associated with glycolysis/ gluconeogenesis, the pentose phosphate pathway, and the lipid metabolism pathway. These data provide evidence that constitutive activation of HIF1α in kidney proximal tubule cells transcriptionally re-programs the regulation of metabolic pathways in a manner similar to that observed in human ccRCC.

Material and methods

Animals

TRACK transgenic positive (TG+) and transgenic negative (TG-) mice were housed five per cage in a 12 hour light/dark cycle in the Research Animal Resource Center of Weill Cornell

Medical College (WCMC). The care and use of animals in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of WCMC.

Whole Transcriptome RNA Sequencing

Total RNA from one thin, outer slice of kidney cortex per kidney was used for whole transcriptome sequencing. Total RNA was extracted using mini-RNAeasy columns (Qiagen, Valencia, CA). The complete transcriptomes of kidney cortices from three γ-HIF1αM3 TRACK (#43 line) TG+ and three γ-HIF1αM3 TG- male C57BL/6 mice (about 13 months old) were sequenced (51-bp single-end reads) on an Illumina HiSeq2000 Sequencer following standard protocols in the Genomics Resources Core Facility at Weill Cornell Medical College. Three lanes were used to sequence all 12 samples (4 samples/lane, 2 kidney samples/mouse). The TRACK RNAseq data has been deposited in the GEO database (accession no. GSE54390, embargoed until publication.).

Data Analysis

Data analysis was mainly performed with the Tuxedo tools software (13). In brief, the RNAseq reads were first aligned to the *Mus musculus* genome (UCSC version mm10) using Tophat version 2.0.6 (13, 14). The aligned reads were assembled into transcripts, their abundance was estimated, and they were tested for differential expression using Cufflinks version 2.1.1 (13, 15). CummeRbund version 2.0.0 (13) was used to analyze the differential expression analysis results. To identify pathways changed in the TRACK TG+ vs TG- kidneys, functional enrichment analysis was performed using the goseq package in R software (16). A stringent threshold in selecting DE genes (FC>3, $q < 0.01$) was used to reduce the false positive ratio. Heatmaps of log2 transformed RPKM values were created in R using the heatmap.2 command of the gplots package.

Human ccRCC data retrieval

Human ccRCC gene expression changes were retrieved from Oncomine (Compendia Bioscience, Ann Arbor, MI) by combining five different datasets of human ccRCC patient samples (17-20). The same five Oncomine datasets of Cancer vs. Normal Analysis of ccRCC that we used in the γ-HIF2αM3 TG+ RNAseq analysis (21) were used in this analysis.

Human ccRCC mRNA data was downloaded from The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/). Only tumor patient data with matched normal and normal patient data with matched tumor were downloaded. All data satisfying this requirement were downloaded, including a total of 470 tumor samples and 68 normal samples. Differential expression between ccRCC and normal kidneys was calculated using the downloaded RPKM (Reads Per Kilobase per Million mapped reads) values. Statistical analyses were performed by student's t-test followed by false discovery rate (FDR)-adjustment (q-value). Statistical significance was defined as $q<0.05$.

Results

Expression of a mutated, constitutively active HIF1α in the proximal tubule (PT) cells of the γ-HIF1αM3 TRACK mice results in early stage tumors morphologically similar to human ccRCC (1). To identify changes in gene expression associated with ccRCC carcinogenesis, we examined the whole transcriptome from cells in TRACK kidney cortex slices compared with transgenic negative (TG-) controls. At the time of sacrifice (∼13 months old), about 50% of the proximal tubules in TRACK mice show clear cell abnormalities. However, further abnormalities, e.g. carcinoma in situ, are not ubiquitously seen in the kidney cortex. The average number of reads per sample was ∼45.6 million, and ∼96% of reads mapped to the *Mus musculus* genome (Supplemental Table 1). A scatter plot of the RPKM values of TRACK TG+ vs TG- kidneys shows that the majority of transcripts evaluated display no change between these two samples (Figure 1a), but there are transcripts that show increased or decreased levels in the TRACK TG+ vs TG- kidneys (Figure 1a). Changes in some of these transcripts have been confirmed by semi-quantitative RT-PCR ((1) and Supplemental Figure 1). Principal component analysis (PCA) shows that there is a clear distinction between TRACK TG+ and TG- kidneys (Figure 1b).

We have shown that the high expression of CA-IX, Glut1, and VEGF proteins in the TRACK kidneys is mainly localized in the clear cell proximal tubules (1). Here we also used immunohistochemistry to examine the protein levels of NDUFA4L2, SLC16A3, and HK2, three of the top genes overexpressed in TRACK kidneys by RNAseq. We detected high expression of NDUFA4L2, SLC16A3, and HK2 primarily in the clear cell proximal tubules (Supplemental Figure 2). These transcripts are also highly expressed in human ccRCC (see next section).

Certain metabolic pathways are over-represented among differentially expressed (DE) genes in TRACK TG+ kidneys

We first examined the over-representation of 274 DE genes (259 overexpressed and 15 underexpressed) in KEGG (Kyoto Encyclopedia of Genes and Genomes). KEGG is a database resource for understanding high-level functions and utilities of biological systems (22, 23). The KEGG PATHWAY is a collection of manually drawn pathway maps of molecular interactions and reaction networks. The 274 DE genes are over-represented in 5 KEGG pathways ($q<0.05$, top 10 pathways are shown in Table 1). Interestingly, seven of the 10 KEGG pathways are related to metabolism and five of these seven KEGG pathways have the lowest p-values in the list (Table 1), indicating that metabolic changes are the most prominent changes in TRACK TG+ kidneys. The most significant KEGG pathway is mmu00010: Glycolysis/Gluconeogenesis ($p=3.87E-06$). The pentose phosphate and peroxisome proliferator-activated receptor (PPAR) signaling pathways are also emphasized.

We also examined the over-representation of DE genes in the Gene Ontology (GO) consortium (24). The GO covers three domains: cellular component, molecular function, and biological process, and we focused on the biological process domain. The 274 DE genes are over-represented in 98 GO biological process ontologies ($q<0.05$, Supplemental Table 2). Similar to the KEGG pathway analysis, seven of the top ten over-represented GO ontologies

are metabolism related, e.g. glucose metabolic process (p=3.57E-10), glucose catabolic process (p=2.36E-08).

Metabolic changes in ccRCC can be explained by gene expression changes observed in TRACK TG+ kidneys

The most significant metabolic change that occurs in ccRCC, and probably in most cancers, is the shift from oxidative phosphorylation of glucose to glycolysis under normoxic conditions, known as the Warburg effect (25). We examined the transcript levels of key genes involved in glycolysis and the TCA cycle in the TRACK TG+ kidneys vs. TG- kidneys (Figure 2). Most glycolysis gene transcripts are increased in the TRACK TG+ kidneys compared to the TG- kidneys, e.g. hexokinase, phosphofructokinase, and pyruvate kinase. TCA cycle genes do not show large decreases in transcript levels in TRACK TG+ kidneys compared to TG- kidneys (FC between 0.8 and 0.9), presumably as a result of the presence of non-transformed cells in the cortex samples. Transcripts encoding pyruvate dehydrogenase kinase, the enzyme that inactivates pyruvate dehydrogenase through phosphorylation (26), are increased in the TRACK TG+ kidneys compared to TG- kidneys (fold change (FC) =7.8). Inactivation of pyruvate dehydrogenase by pyruvate dehydrogenase kinase prevents the conversion of pyruvate to acetyl-CoA (26), which is oxidized in mitochondria in the TCA cycle. The lower level of acetyl-CoA thereby inhibits the TCA cycle. Consistent with this, the transcript for lactate dehydrogenase, which converts pyruvate to lactate, is increased in the TRACK TG+ relative to TG- kidneys. All of these changes indicate an important role for HIF1α in changing the metabolism of kidney cells to glycolysis under normoxic conditions. Heatmaps created from genes involved in glycolysis and in the TCA cycle also confirmed these changes (Figure 3).

The TRACK/HIF1α kidney transcriptome shows similarities with the human ccRCC transcriptome

Using a fold-expression change of >2 or < 0.5 and q <0.05, 655 genes showed increased mRNA levels and 55 genes showed decreased mRNA levels in TRACK TG+ kidney samples compared with the TG- control kidney cortex samples. As expected, several HIF1α target genes were highly expressed at the transcript level in TRACK kidney samples, including hexokinase 2 (HK2, 70X), carbonic anhydrase IX (CA-IX, 9.3X), glucose transporter 1 (Glut-1 or Slc2a1, 4.8X) etc, which were confirmed by semi-quantitative RT-PCR ((1) and Supplemental Figure 1).

We first compared the expression profile of human ccRCC, as reported in the Oncomine database (27), with our TRACK+ expression profile. We identified the 20 genes most highly overexpressed and 20 genes most highly underexpressed at the RNA level in human ccRCC by combining five different datasets of human ccRCC patient samples (17-20). The total number of ccRCC patients in all five data sets is 175. Five of the 20 genes most highly overexpressed in human ccRCC were similarly expressed in TRACK TG+ kidneys (FC>2) (Table 2): NDUFA4L2, C7ORF68, EGLN3, SLC16A3, and CA-IX. None of the 20 genes highly underexpressed in human ccRCC shows significant downregulation in our TRACK TG+ kidneys (FC<0.5) (Table 2). However, the numbers of genes that show reduced

expression in TRACK TG+ vs TG- kidneys is most likely an underestimate because only 30-50% of PTs in TRACK TG+ kidneys demonstrate morphologic changes (1).

We also compared the expression profile of human ccRCC mRNA downloaded from the TCGA database with our TRACK+ expression profile. We identified the 20 genes most highly overexpressed and the 20 genes most highly underexpressed at the RNA level in human ccRCC from TCGA data. Five of the 20 genes most highly overexpressed in human ccRCC were similarly expressed in TRACK TG+ kidneys (FC>2) (Table 3). None of the 20 genes highly underexpressed in human ccRCC shows significant downregulation in our TRACK TG+ kidneys (FC<0.5) (Table 3).

We then performed the reverse comparison by identifying the top genes over- or under-expressed at the RNA level in the TRACK TG+ vs. TG- WT kidneys and comparing these transcripts to those in the TCGA database and the combined Oncomine human ccRCC datasets. Eleven of the 20 genes highly overexpressed in TRACK TG+ kidneys show overexpression (FC>2) in the TCGA data (Table 4). Ten of the 20 genes highly underexpressed in TRACK TG+ kidneys show underexpression (FC<0.5) in the TCGA dataset (Table 4). Four of the 20 genes highly overexpressed in TRACK TG+ kidneys show overexpression (FC>2) in the combined Oncomine datasets (Table 4). Eight of the 20 genes highly underexpressed in TRACK TG+ kidneys show underexpression (FC<-2) in the combined Oncomine datasets (Table 4). We conclude from analysis of these data that expression of a mutant, constitutively active HIF1α protein in kidney PTs results in a transcriptome that partially resembles those of human ccRCCs.

Discussion

We previously established the TRACK model, which mimics early stage human ccRCC through expression of a mutated, constitutively active human HIF1α construct in the PT cells (1). Here we present genome-wide transcriptome analysis of the TRACK TG+ kidney cortex cells, which are mainly PT cells. We identified 655 up-regulated genes and 55 down-regulated genes that are differentially expressed in the TRACK TG+ kidneys compared to the TG- kidneys (FC>2). Some of these genes also show increased or decreased transcript levels in human ccRCC specimens compared to normal kidneys, respectively (Table 4). For example, NDUFA4L2 is the gene overexpressed to the greatest extent in the human ccRCC datasets in Oncomine. The median fold change in human ccRCC compared to normal kidney from all 5 datasets is 53.9 (Table 2). Similarly, NDUFA4L2 transcript levels are about 68 fold higher in TRACK TG+ kidneys compared to TG- kidneys (Table 2). Similarities can also be seen in genes that are down-regulated in TRACK TG+ vs. TG- and human ccRCC vs. normal kidneys, e.g. 4-hydroxyphenylpyruvic acid dioxygenase (HPD) (Table 4). These results suggest that the transcriptome of the TRACK TG+ kidneys partially resembles that of human ccRCC cells. Furthermore, we have examined the expression patterns of some of these top genes overexpressed in the TRACK kidneys by immunohistochemistry ((1) and Supplemental Figure 2). The high expression of these proteins occurs primarily in the clear cell proximal tubules. These data support our contention that the changes in the transcriptome of TRACK kidneys that we have seen by RNAseq are mainly, if not all, caused by changes in these clear cell proximal tubules.

New evidence suggests that ccRCC is a metabolic disease (6, 7). All known kidney cancer susceptible genes are involved in the regulation of metabolic pathways (6). ccRCC cells contain increased amounts of glycogen and lipid in their cytoplasm (4, 5). Increased glycogen and lipid are also seen in the TRACK TG+ kidney cells (1). Our analysis of the entire transcriptome of the TRACK TG+ kidney PT cells identified some altered metabolic pathways, including increased transcript levels of genes involved in glycolysis/gluconeogenesis (Figure 2A) and decreased transcript levels of genes involved in the TCA cycle (Figure 2B). Furthermore, increased mRNA levels of the protein pyruvate dehydrogenase kinase can inactivate pyruvate dehydrogenase, which converts pyruvate to acetyl-CoA (26). Decreased levels of acetyl-CoA, together with decreased transcript levels of genes involved in the TCA cycle, should result in decreased activity of the TCA cycle. The products of the TCA cycle, NADH and succinate, are used in the oxidative phosphorylation pathway. Decreased TCA cycle activity and decreased levels of NADH and succinate should result in lower levels of substrates, and as a result, decreased oxidative phosphorylation. This phenotype recapitulates much of what is described for the Warburg effect in tumor cells (25). HIF1α, rather than HIF2α, is the main regulator of these pathways (28, 29), further emphasizing the importance of HIF1α in modulating the metabolic alterations in ccRCC.

The histone deacetylase, Sirtuin-6 (SIRT6), can suppress the transcription of the HIF1α gene (30). Increased glucose uptake and increased HIF1α activity were shown in SIRT6-deficient cells and mice and this increase in HIF1α activity was sufficient to cause tumorigenesis (30). SIRT6 has been reported to be a potential tumor suppressor gene (31, 32). Thus, either the absence of VHL or the lack of SIRT6 can results in an increase in HIF1α activity and tumorigenesis. In summary, our results suggest that constitutive activation of HIF1α in kidney PT cells in our TRACK model induces a phenotype similar to the tumor phenotype associated with the Warburg effect (25).

In addition to inactivating mutations in the VHL gene, inactivating mutations in other genes, e.g. PBRM1 (33), SETD2 (34), etc., are commonly seen in patients with advanced ccRCC (35-38). Mutations and/or changes in the expression of these genes probably play important roles in the development of advanced ccRCC. This might explain why TRACK mice only mimic early stage ccRCC. This hypothesis is being tested in the TRACK mouse model by mutation/knockout of additional genes, such as PBRM1 and SETD2.

Importantly, the transgenic mice we generated that overexpress a constitutively active HIF2α do not show major transcript changes that reflect those in human ccRCC (21). Thus our data reported here and those of many other researchers (reviewed in 12) indicate that the activity of HIF1α is more closely linked to ccRCC tumorigenesis than the activity of HIF2α.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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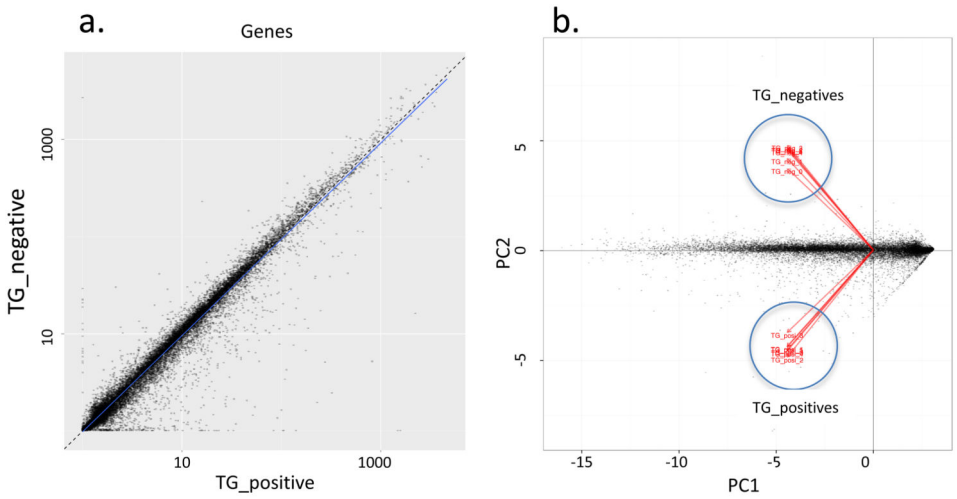

Figure 1. Global plotting of TRACK TG+ vs TG- kidney RNAseq result

A scatter plot of the RPKM values between TRACK TG+ vs. TG- kidneys (a) and a principal component analysis result (b) are shown. The majority of transcripts show no differences in levels between TRACK TG+ vs. TG-. There are transcripts that show increased (dots in the bottom right part of A) or decreased (dots in the top left part of A) levels in the TRACK TG+ vs TG- kidneys. Principal component analysis (PCA) shows that there is a clear distinction between TRACK TG+ and TG- kidneys (b).

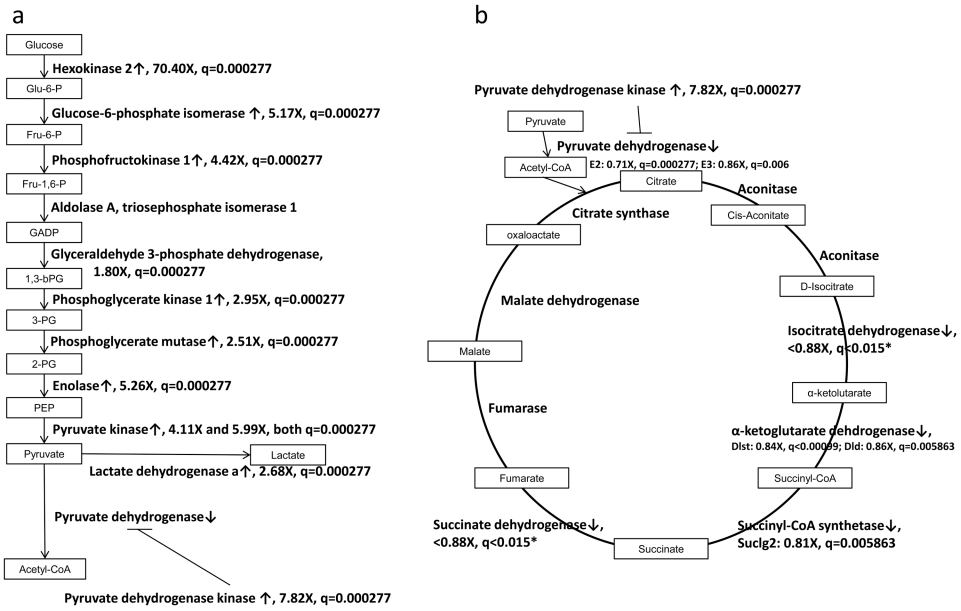

Figure 2. Transcript changes for glycolysis and TCA cycle transcripts in TRACK+/TG- kidney cells

Simplified pathway maps of glycolysis (a) and the TCA cycle (b) with mRNA changes shown. Un-boxed inputs are the gene names. Genes that are over-expressed (FC>2) in the TRACK TG+ kidneys are indicated by ↑, while genes that are under-expressed (FC<0.9) in the TRACK TG+ kidneys are indicated by ↓. Since the under-expressed genes are potentially under-estimated in the RNAseq results, the fold change threshold for under-expressed genes is set at <0.90. * Both isocitrate dehydrogenase (IDH) and succinate dehydrogenase (SDH) complexes contain more than 2 transcripts that are significantly decreased in TRACK TG+ kidneys. The fold change and q-value shown are the least significant ones in these two complexes.

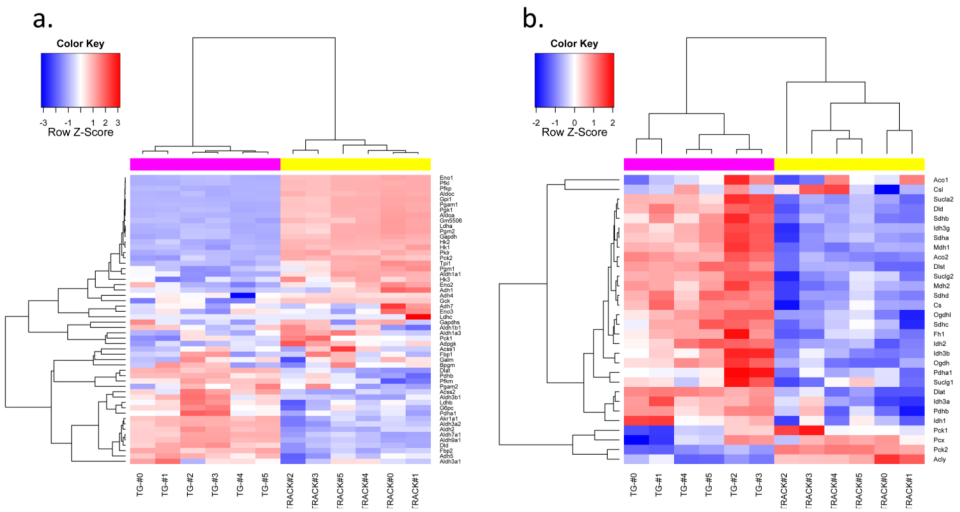

Figure 3. Heatmaps of transcripts encoded by genes involved in glycolysis and the TCA cycle, TRACK+/TG-

Heatmaps of genes involved in glycolysis (a) and the TCA cycle (b). Log2 transformed RPKM values of 61 and 31 genes involved in glycolysis and TCA cycle pathways were used to create these heatmaps. The animal identity is indicated by the colored rows on top of the heatmap matrix. The magenta color indicates TRACK TG+ mice. The yellow color indicates TRACK TG- mice. The blue color in the heatmap matrix indicates relatively decreased transcript levels and the red color indicates relatively increased transcript levels compared to the mean transcript level for each gene. Brighter blue or red color indicates a greater fold change. The glycolysis genes listed in Figure 2 show increased transcript levels in the TRACK TG+ kidneys (red color) compared to TG- kidneys (a). The TCA cycle genes generally show decreased transcript levels in the TRACK TG+ kidneys (blue colored) compared to TG- kidneys (b).

Table 1

Ten most significant KEGG pathways that are over-represented among the differentially expressed (DE) genes

p_value	q_value	KEGG_ID	KEGG_pathway
3.87E-06	8.70E-04	mmu00010	Glycolysis / Gluconeogenesis
2.27E-05	2.55E-03	mmu00051	Fructose and mannose metabolism
2.36E-04	1.77E-02	mmu03320	PPAR signaling pathway
6.70E-04	3.77E-02	mmu00052	Galactose metabolism
8.91E-04	4.01E-02	mmu00030	Pentose phosphate pathway
1.40E-03	5.24E-02	mmu04060	Cytokine-cytokine receptor interaction
2.59E-03	8.32E-02	mmu00670	One carbon pool by folate
3.50E-03	8.97E-02	mmu01100	Metabolic pathways
3.59E-03	8.97E-02	mmu00750	Vitamin B6 metabolism
4.27E-03	9.60E-02	mmu04672	Intestinal immune network for IgA production

Table 2

Twenty genes that show the highest fold increases in transcript levels in human ccRCC compared to normal human kidney from the Oncomine database

Oncomine Median Rank	Oncomine p-Value	Oncomine Median Fold Change	Gene	fold-change TRACK TG+/TG-	p-value	q-value
Increased in ccRCC						
7	5.55E-23	53.9	NDUFA4L2	67.92514	5e-05	2.77e-04
14	3.91E-13	16.5	C7ORF68	2.824433	5e-05	2.77e-04
14	2.82E-11	12.5	ENO2	1.668066	1e-04	5.27e-04
16	9.50E-19	12.2	EGLN3	30.98842	5e-05	2.77e-04
21	1.73E-14	11.0	IGFBP3	1.572941	5e-05	2.77e-04
25	1.23E-10	12.2	SPAG4	1.174346	1	1
28	2.83E-17	15.0	AHNAK2	Not found		
30	3.79E-7	8.4	TMCC1	1.04368	0.60145	0.754576
36	2.19E-16	8.6	RNASET2	1.048042	0.62605	0.774746
38	1.93E-13	8.2	CAV1	1.385522	5e-05	2.77e-04
41	2.54E-13	7.1	SLC16A3	114.6301	5e-05	2.77e-04
46	6.8E-10	4.6	LCP2	1.774849	5e-05	2.77e-04
47	6.92E-16	18.0	CA-IX	9.340484	5e-05	2.77e-04
48	7.84E-10	15.4	NETO2	1.513053	1	1
49	8.25E-10	2.6	IFNGR2	1.242553	1e-04	5.27e-04
57	3.12E-15	4.9	ABCG1	1.500543	5e-05	2.77e-04
57	1.37E-12	2.3	UBE2L6	1.62286	5e-05	2.77e-04
59	1.59E-10	3.2	DIAPH2	1.001564	0.97995	0.988178
59	1.50E-9	2.4	ALDOA	1.840662	5e-05	2.77e-04
59	1.69E-8	3.4	SLC15A4	1.107461	0.28295	0.453989
Decreased in ccRCC						
4	1.41E-019	-283.867	UMOD	1.038859	0.5042	0.675799
4	2.63E-019	-44.184	SFRP1	1.029511	0.5378	0.705209
22	1.38E-012	-6.616	TFCP2L1	1.343029	5e-05	2.77e-04
36	1.40E-006	-72.014	SLC12A1	1.020973	0.9791	0.987744
41	9.48E-011	-14.893	GATA3	1.078646	0.2454	0.410806

Oncomine Median Rank	Oncomine p-Value	Oncomine Median Fold Change	Gene	fold-change TRACK TG+/TG-	p-value	q-value
44	3.25E-012	-51.115	CLDN8	1.038964	0.44125	0.618216
44	6.68E-011	-20.454	LPPR1	No mouse homolog		
45	2.75E-013	-10.533	ALDH6A1	0.7895559	5e-05	2.77e-04
45	3.32E-012	-223.376	CALB1	1.019128	0.69485	0.821341
47	4.80E-013	-8.431	DCN	2.206112	5e-05	2.77e-04
51	3.67E-010	-47.197	ATP6V0A4	0.7976494	1e-04	5.27e-04
51	2.65E-006	-172.954	KNG1	1.283858	0.10715	0.217875
54	1.82E-012	-34.705	ALDOB	HIDATA	1	1
56	2.08E-012	-7.697	TCF21	1.3445	4e-04	1.86e-03
57	3.71E-006	-17.206	MAL	1.11156	0.02155	0.0601907
61	7.17E-010	-16.262	PTH1R	0.7618173	5e-05	2.77e-04
65	1.17E-009	-3.402	GABARAPL3	No mouse homolog		
68	1.45E-009	-3.413	PROSAPIP1	0.6268898	5e-05	2.77e-04
77	2.44E-009	-50.953	KCNJ1	1.030991	0.51935	0.689311
79	1.87E-011	-17.438	ERBB4	1.001307	0.9846	0.990418

The list of the top 20 genes that show elevated or decreased transcript levels in human ccRCC was retrieved from Oncomine by combining five different datasets of human ccRCC patient samples, totaling 175 patients. These five datasets are the same as those discussed in (21). The fold changes in mRNA levels in these genes in TRACK TG+ mice versus TG- kidneys are listed.

Table 3

Twenty genes that show the highest fold increases or decreases in transcript levels in human ccRCC compared to normal human kidney from TCGA

TCGA q-value	TCGA Fold Change	Gene	fold-change TRACK TG+/TG-	p-value	q-value
Increased in ccRCC					
2.85E-02	140.62	HP	4.47	5.00E-05	2.77E-04
2.59E-10	81.87	GUCA2B	0.88	7.30E-03	2.41E-02
1.74E-122	54.30	CA9	9.34	5.00E-05	2.77E-04
6.09E-28	51.35	FABP7	0.82	1.47E-02	4.36E-02
1.21E-23	47.63	MCHR1	0.69	2.35E-03	9.01E-03
3.19E-02	45.56	HHATL	0.88	8.02E-02	1.73E-01
3.06E-105	44.88	NDUFA4L2	67.93	5.00E-05	2.77E-04
6.80E-48	44.07	HSF4	1.78	5.00E-05	2.77E-04
1.82E-45	34.72	KISS1R	1.06	9.80E-01	9.88E-01
9.17E-61	34.31	CYP2J2	0.93	8.85E-01	9.38E-01
2.65E-41	31.87	PNCK	1.71	8.10E-03	2.63E-02
2.15E-33	31.45	APOC1	1.62	4.45E-02	1.08E-01
5.89E-17	29.99	CD5L	0.39	5.00E-05	2.77E-04
2.20E-110	29.31	ANGPTL4	0.76	5.00E-05	2.77E-04
1.34E-94	28.73	COL23A1	1.34	2.23E-02	6.19E-02
2.92E-06	24.59	LBP	1.73	5.00E-05	2.77E-04
1.39E-91	21.66	ENPP3	0.99	9.10E-01	9.52E-01
7.13E-94	20.73	C7orf68	2.82	5.00E-05	2.77E-04
2.51E-26	19.66	KCNN1	0.78	1.07E-02	3.34E-02
5.94E-44	19.58	NXPH4	10.02	5.00E-05	2.77E-04
Decreased in ccRCC					
9.67E-16	0.002	AQP2	1.260	5.00E-05	2.77E-04
3.20E-14	0.002	UMOD	1.039	5.04E-01	6.76E-01
5.23E-16	0.003	SLC12A1	1.021	9.79E-01	9.88E-01
5.73E-13	0.004	TMEM207	0.888	2.22E-01	3.80E-01
3.09E-11	0.005	FXYD4	1.055	4.58E-01	6.34E-01

TCGA q-value	TCGA Fold Change	Gene	fold-change TRACK TG+/TG-	p-value	q-value
6.23E-12	0.006	CALB1	1.019	6.95E-01	8.21E-01
2.95E-12	0.008	NPHS2	1.249	5.00E-05	2.77E-04
1.70E-18	0.009	DUSP9	1.043	5.90E-01	7.47E-01
1.18E-11	0.009	SOST	1.091	4.90E-01	6.64E-01
1.07E-20	0.013	KNG1	1.284	1.07E-01	2.18E-01
5.63E-21	0.013	HS6ST2	1.724	5.00E-05	2.77E-04
5.46E-12	0.014	DDN	1.071	3.69E-01	5.48E-01
3.20E-17	0.014	CLDN16	1.068	4.52E-01	6.29E-01
1.07E-24	0.015	KCNJ1	1.066	8.46E-01	9.18E-01
1.07E-24	0.015	KCNJ1	1.031	5.19E-01	6.89E-01
1.05E-13	0.015	ESRRB	0.884	4.01E-02	9.96E-02
6.04E-24	0.016	KCNJ10	1.113	2.36E-02	6.49E-02
1.22E-09	0.016	SLC22A8	0.549	5.00E-05	2.77E-04
4.49E-24	0.016	TFAP2B	1.055	3.23E-01	4.99E-01
3.08E-21	0.017	RALYL	1.488	2.81E-02	7.49E-02

The list of the top 20 genes that show elevated or decreased transcript levels in human ccRCC was retrieved from TCGA. The fold changes in mRNA levels in these genes in TRACK TG+ mice versus TG- kidneys are listed.

Table 4

Twenty genes that show the greatest increases or decreases in transcript levels in the TRACK TG+ vs. WT kidneys by RNAseq

fold-change TRACK TG+/TG-	p-value	q-value	Gene	TCGA Human ccRCC/normal kidney fold change	Oncomine Human ccRCC/normal kidney median fold change
Increased in TRACK					
260.2342364	5e-05	2.77e-04	F2	11.76	1.008
241.0075905	5e-05	2.77e-04	IGFBP2	0.29	-5.309
212.4951738	5e-05	2.77e-04	GRIN1	2.67	1.342
208.5679355	5e-05	2.77e-04	AIRE	2.06	-1.075
196.9580885	5e-05	2.77e-04	APOA4	244.29	-1.227
158.8470346	5e-05	2.77e-04	RBP4	1.11	-1.13
148.1545998	5e-05	2.77e-04	APOC3	3.61	-1.815
127.8628806	5e-05	2.77e-04	A2M	1.16	1.809
114.6301281	5e-05	2.77e-04	SLC16A3	8.15	7.132
97.86820107	5e-05	2.77e-04	ADAM7	7.79	1.07
86.29550828	5e-05	2.77e-04	ZFP92	0.74	-0.0815
78.7336936	6e-04	0.00266966	CRISP1	0.21	-1.003
77.00377581	5e-05	2.77e-04	AREG	1.43	1.312
70.96599132	5e-05	2.77e-04	RASGRF2	1.10	1.568
70.39792583	5e-05	2.77e-04	NLRP10	0.29	-1.286
70.39041145	5e-05	2.77e-04	HK2	10.17	13.568
67.9251413	5e-05	2.77e-04	NDUFA4L2	44.88	53.935
50.96140222	0.00015	0.000762465	CPB2	8.97	1.035
42.27652553	5e-05	2.77e-04	ANKRD37	2.15	3.846
38.17346108	5e-05	2.77e-04	DKK1	1.51	-1.058
Decreased in TRACK					
0.03993031	0.0145	0.0431824	DIO3OS	5.18	-1.025
0.042320553	5e-05	2.77e-04	SOX2OT	0.38	-2.8495
0.112181952	0.0017	0.0067467	KRT13	0.37	-1.020
0.122714679	5e-05	2.77e-04	HPD	0.04	-79.57
0.196070105	5e-05	2.77e-04	IDO2	1.58	1.019
0.215859171	5e-05	2.77e-04	C4A	3.40	2.176

fold-change TRACK TG+/TG-	p-value	q-value	Gene	TCGA Human ccRCC/normal kidney fold change	Oncomine Human ccRCC/normal kidney median fold change
0.218647669	5e-05	2.77e-04	HRG	0.24	-14.081
0.251872656	5e-05	2.77e-04	AZGP1	0.29	-12.585
0.312454798	5e-05	2.77e-04	NLRC4	3.32	-1.0665
0.31642082	5e-05	2.77e-04	PZP	1.14	1.012
0.327772061	5e-05	2.77e-04	CTXN3	0.10	-7.343
0.331900011	5e-05	2.77e-04	ANXA13	3.27	1.971
0.361353874	5e-05	2.77e-04	UPP2	0.04	-5.804
0.378206047	5e-05	2.77e-04	APOH	1.23	-6.871
0.39051491	5e-05	2.77e-04	CD5L	29.99	-1.017
0.409784094	5e-05	2.77e-04	TMEM28	0.60	-1.095
0.426331824	5e-05	2.77e-04	CNDP2	1.59	1.185
0.42792761	5e-05	2.77e-04	FCAMR	0.37	-2.2115
0.429718771	0.0156	0.0459592	ANGPTL7	0.34	-1.049
0.436898506	0.0156	0.0459592	GPT	0.71	-1.344

The list of the top 20 genes that show elevated or decreased transcript levels in the TRACK TG+ vs. TG- kidneys was compiled from the RNAseq results. The fold changes in mRNA levels of these genes in human ccRCC were compiled from Oncomine (median fold changed are used here) by combining five different sets of human ccRCC patient samples (see Table 1 for more information) or from TCGA. Genes that have no measurements in all five datasets were excluded from this list.