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An integrated analysis of mRNA-miRNA transcriptome data revealed hub regulatory networks in three genitourinary cancers

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Abstract: Bladder, kidney, prostate and testicular carcinoma are the top four genitourinary cancers in China. Here we analyzed mRNA and miRNA expression profiles of carcinomas of the bladder (TCC), kidney (ccRCC) and testis (TGCT) to uncover their specific regulatory mechanisms. The gene expression profiles of GSE31617 were downloaded from GEO database, which contained 27 samples, including 10 TCC, 7 TGCT and 10 ccRCC. Specific up- and down-regulated differentially expressed genes (DEGs) and differentially expressed microRNAs (DEmiRNAs) of each cancer were selected and target genes of DEmiRNAs were predicted. Gene interaction network of the shared genes and target genes of DEmiRNAs of each cancer was predicted by STRING and constructed by Cytoscape. In each cancer, we build regulatory networks of hub genes selected and conducted GO analysis of enriched genes. Furthermore, we chose four hub genes (*SALL4*, *RHEB*,*CDC42* and *TNN*) for survival analysis in OncoLnc database, and they all had effects on the survival rate of another genitourinary cancer-kidney renal clear cell carcinoma (KIRC). In conclusion, the present study indicated that the identified hub genes promote our understanding of molecular mechanisms underlying the development of three genitourinary cancers, and might be used as molecular targets and diagnostic biomarkers for the treatment of them.

Introduction

Genitourinary cancers show huge effects on human health all over the world, of which bladder, kidney, prostate and testicular carcinoma are the top four genitourinary cancers in China (Gu *et al.,* 2002), while prostatic carcinoma, bladder and kidney carcinoma are the top three genitourinary cancers in America (Siegel *et al.*, 2016), other genitourinary cancer types are relatively uncommon. However, bladder cancer has become a common cancer globally, with estimated 430 000 new cases diagnosed in 2012, and bladder cancer ranks as the ninth most frequently-diagnosed cancer worldwide, with the highest incidence rates observed in men in Southern and Western Europe, North America, as well in certain countries in Northern Africa or Western Asia (Antoni *et al.,* 2017). RCC is the eighth most common cancer in the USA. The estimated numbers of new RCC cases and deaths in the USA for 2013 are 65 150 and 13 680, respectively, with a worldwide annual increase of 1.5–5.9% (Liu *et al.*, 2016; Azer *et al.,* 2017). Testicular cancer is treatable and the cure rate is approximately 95%. It is most common in men between the ages of 15 and 35. While early detection, diagnosis, and treatment are all important factors for treating the disease, fertility and quality of life are also important issues to address in patients with testicular cancer (Albers *et al.,* 2015).

The high-throughput platforms for analysis of gene/miRNA expression, such as RNA-seq of transcriptome, exome and whole transcriptome, are increasingly valued as promising tools in medical oncology with great clinical applications (Ord *et al.,* 2005; Ramsey *et al.,* 2017). There are also a number of studies of genitourinary cancers by high-throughput platforms: An integrative analysis of extracellular and intracellular bladder cancer cell line proteome with transcriptome resulted in 253 "verified" proteins based on the agreement of at least 2 strategies, which improving coverage and validity of –omics' findings (Latosinska *et al.,* 2016); 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 the cancer genome atlas databases showed that constitutive activation of HIF1 alpha in kidney proximal tubule cells transcriptionally reprograms the regulation of metabolic pathways in the kidney and that HIF1a is a major contributor to the altered metabolism observed in human ccRCC (Fu *et al.*, 2015); a comparative mRNA and microRNA expression profiling of three genitourinary cancers(ccRCC,TCC and TGCT) reveals

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common hallmarks and cancer-specific molecular events (Li *et al.*, 2011).

miRNAs are endogenous small non-coding RNAs (~22 nucleotides) that modulate gene expression at the post-transcriptional level by binding to the 3' untranslated region (3' -UTR) of target mRNAs(Nelson *et al.*, 2003). Recent studies have shown that some miRNAs are involved in various cancers (Andres-Leon *et al.*, 2017; Li *et al.*, 2017a; Paul *et al.*, 2017). In the present study, we downloaded the original data (GSE31617) from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/), which is a comparative mRNA and microRNA expression profiling of three genitourinary cancers (ccRCC,TCC and TGCT) (Li *et al.*, 2011). We focused on specific up and down-regulated DEGs and DEmiRNAs of each cancer were selected and target genes of up and down-regulated miRNAs were predicted by Targetscan. Gene interaction network of the shared genes of up or down-regulated differentially DEGs and target genes of DEmiRNAs of each cancer was predicted by STRING and constructed by Cytoscape software. In each cancer, we build regulatory networks of hub genes selected and conducted GO analysis of enriched genes. Furthermore, we chose four hub genes for survival analysis in OncoLnc data base. By identifying hub genes and their interaction network, we may get further insight in genitourinary cancers and may disclose molecular targets and diagnostic biomarkers for treatment.

Materials and methods

Sequencing data

The gene/miRNA expression profiles of GSE31617 were downloaded from GEO database. GSE31617 were sequenced using the Illumina GAII platform according to manufacturer's instructions (Illumina Inc., USA). The GSE31617 dataset contained 27 samples, including 10 TCC, 7 TGCT and 10 ccRCC samples.

Target prediction of differentially expressed miRNAs

The biological relevance of differentially expressed miRNAs was analyzed through their target genes. The target gene set was generated TargetScan Release 7.1 (http://www.targetscan.org/vert_71/). And then, putative targets were subjected to further analysis.

Interaction and GO analysis of differentially expressed genes and miRNA targets

Venn diagrams were drawn in the website of Bioinformatics & Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/). The DEGs and DEmiRNA target genes interaction network was illustrated by STRING (http://string-db.org/) and Cytoscape 3.1.0 (http://www.cytoscape.org/). GO analysis was generated in STRING. Moreover, the gene with the absolute value of log2 ratio≥1 and FDR≤0.01 was designated the DEGs. Statistical analyses were conducted with SPSS 10.0.

Illustration of survival curves

Survival data was downloaded in OncoLnc database (http://www.oncolnc.org/) with the criteria of lower percentile 15/upper percentile 15 and logrank *p* value≤0.005, as there were no data for three cancers we analyzed in the database, we used the data of kidney renal clear cell carcinoma (KIRC), which is also one of genitourinary cancers. Four genes in the hub interaction network of TGCT, TCC and ccRCC were selected with affected survival rate.

Results

Identification of differentially expressed mRNAs/miRNAs (DEGs/DEmiRNAs) generated in TGCT, TCC and ccRCC

A total number of 10 TCC, 7 TGCT and 10 ccRCC samples were analyzed in this study. Using the criteria of absolute value of log2 ratio≥1 and FDR≤0.01, a total of 3181, 4317 and 4592 DEGs were identified in TGCT, TCC and ccRCC, respectively, after the analyses of GSE31617. Using the same criteria and dataset, a total number of 220, 194 and 105 DEmiRNAs were identified in TGCT, TCC and ccRCC, respectively.

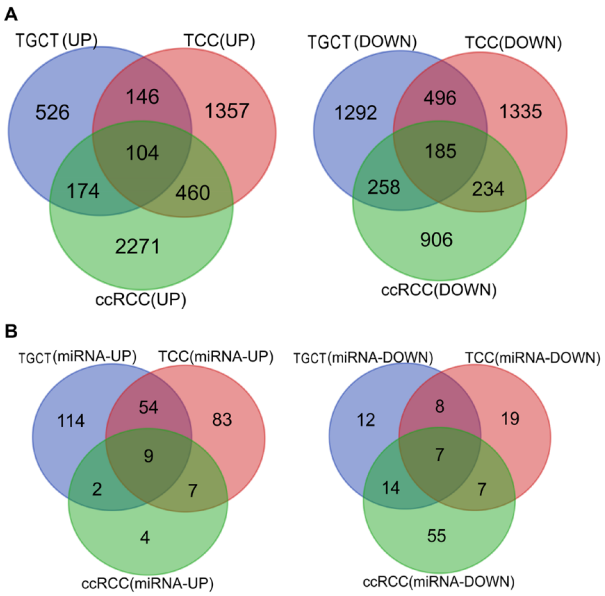


FIGURE 1. Venn diagram of DEGs and DEmiRNAs. (A)Venn diagram of up-regulated (left) and down-regulated (right) DEGs in each cancer. (B)Venn diagram of up-regulated (left) and down-regulated (right) DEmiRNAs in each cancer.

We focused on the DEGs and DEmiRNAs that were specifically expressed in TGCT, TCC and ccRCC. As shown in Fig. 1, TGCT showed the least number of specific up-regulated DEGs and most abundant number of specific up-regulated DEmiRNAs, while ccRCC showed the most abuntant number of specific up-regulated DEGs and least number of specific up-regulated DEmiRNAs (Figs. 1 (A-B)). On the contrary, TGCT showed the most abundant number of specific down-regulated DEGs and least number of specific down-regulated DEmiRNAs, while ccRCC showed the least number of specific down-regulated DEGs and most abundant number of specific down-regulated DEmiRNAs (Figs. 1A-1B).

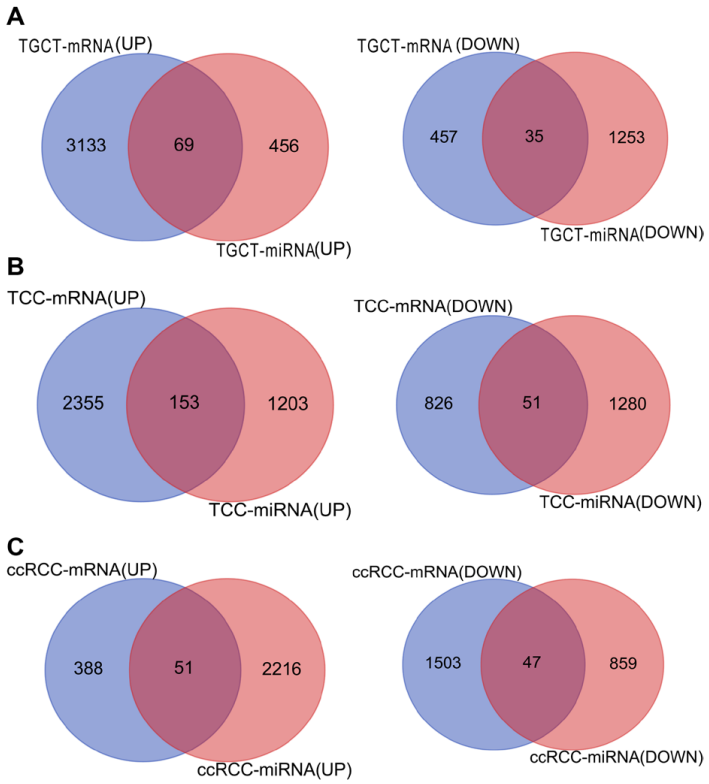
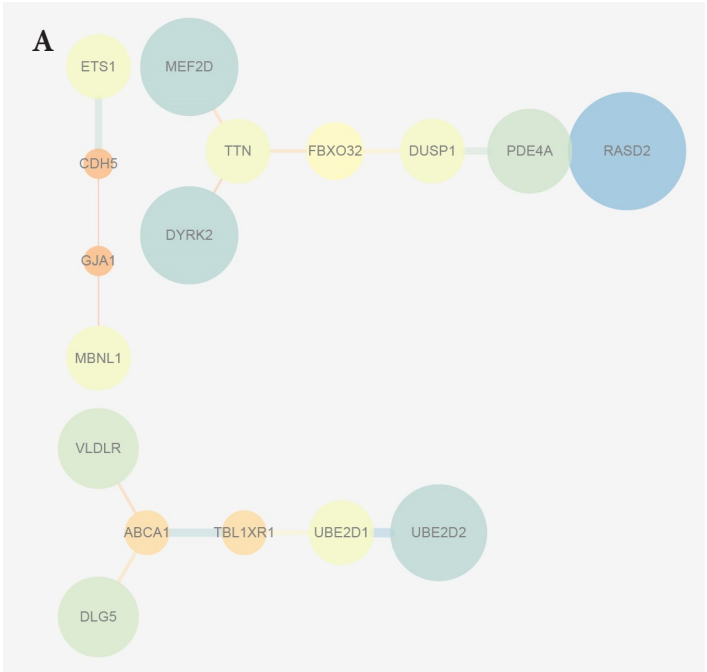


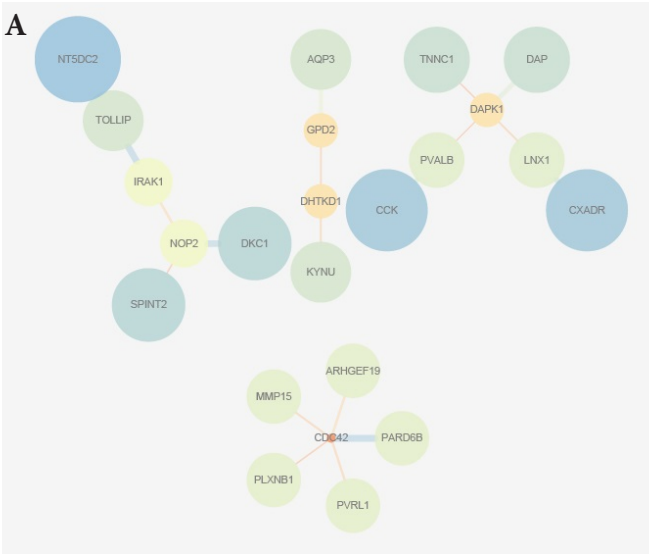
FIGURE 2. Venn diagram of DEGs and target genes of DEmiRNAs. (A) Venn diagram of DEGs and target genes of DEmiRNAs in TGCT. (B) Venn diagram of DEGs and target genes of DEmiRNAs in TCC. (C) Venn diagram of DEGs and target genes of DEmiRNAs in ccRCC.



B

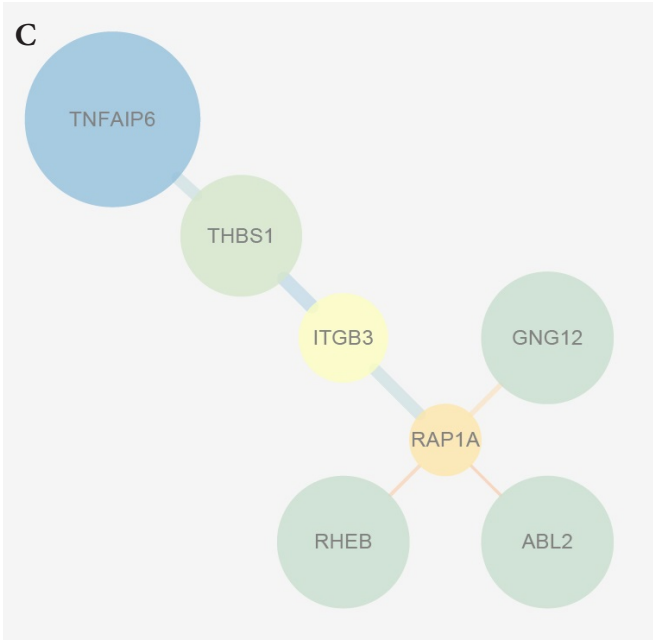
Term	PValue
negative regulation of gene expression	5.76E-04
regulation of cAMP-mediated signaling	0.013918286
fat pad development	0.016679103
response to laminar fluid shear stress	0.019432355
regulation of apoptotic process	0.021673386
locomotory behavior	0.023109315
negative regulation of DNA biosynthetic process	0.033085836
adult heart development	0.035794117
negative regulation of transcription regulatory region DNA binding	0.038494974
nervous system development	0.046159631

FIGURE 3. Interaction and GO analysis of common genes from DEGs and target genes of DEmiRNAs in ccRCC. (A) Interaction gene regulatory network of up-regulated DEGs and target genes of DEmiRNAs in ccRCC. (B) GO analysis of up-regulated DEGs and target genes of DEmiRNAs in ccRCC.



B

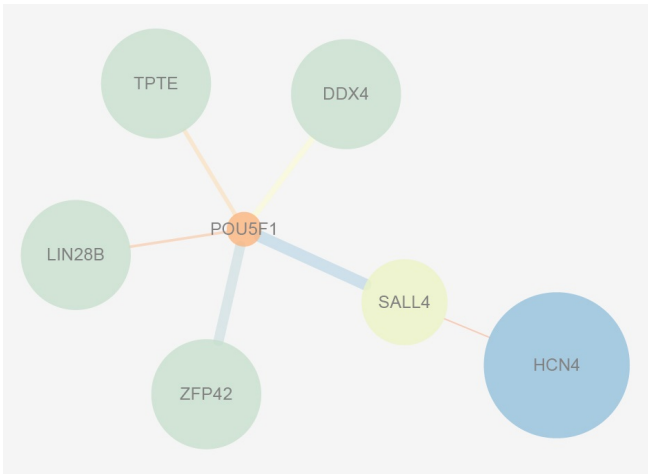
Term	PValue
establishment or maintenance of cell polarity	0.0011910
protein localization to plasma membrane	0.0108249
calcium ion homeostasis	0.010998
epithelial cell differentiation	0.0143862
negative regulation of transcription, DNA-templated	0.0291791
ER to Golgi vesicle-mediated transport	0.0293430
Wnt signaling pathway, planar cell polarity pathway	0.0294397



D

Term	PValue
small GTPase mediated signal transduction	0.023433496
muscle contraction	0.029351176
positive regulation of macrophage activation	0.029614391
leukocyte migration	0.037317439
mitochondrial fusion	0.044098781
cell cycle arrest	0.048447332

FIGURE 4. Interaction and GO analysis of common genes from DEGs and target genes of DEmiRNAs in TCC. (A) Interaction gene regulatory network of up-regulated DEGs and target genes of DEmiRNAs in TCC. (B) GO analysis of up-regulated DEGs and target genes of DEmiRNAs in TCC. (C) Interaction gene regulatory network of down-regulated DEGs and target genes of DEmiRNAs in TCC. (D) GO analysis of down-regulated DEGs and target genes of DEmiRNAs in TCC.



B

Term	PValue
regulation of ion transmembrane transport	0.004910048
multicellular organism development	0.022138654
potassium ion import across plasma membrane	0.027534149

FIGURE 5. Interaction and GO analysis of common genes from DEGs and target genes of DEmiRNAs in TGCT. (A) Interaction gene regulatory network of up-regulated DEGs and target genes of DEmiRNAs in TGCT. (B) GO analysis of up-regulated DEGs and target genes of DEmiRNAs in TGCT.

Integrated analysis of DEGs and the target genes of DEmiRNAs in TGCT, TCC and ccRCC

In order to identify the interaction of DEGs and DEmiRNAs, we predicted target genes of DEmiRNAs as a bridge. Target genes of DEmiRNAs were predicted through TargetScan Release 7.1 (http://www.targetscan.org/vert_71/). We identified 525, 1356 and 2267 target genes of up-regulated DEmiRNAs and 1288, 1331 and 906 target genes of down-regulated DEmiRNAs in TGCT, TCC and ccRCC, respectively (Data not shown).

We then combined up-regulated DEGs with target genes of up-regulated DEmiRNAs and down-regulated DEGs with target genes of down-regulated DEmiRNAs in TGCT, TCC and ccRCC (Fig. 2).The largest number of shared up- or down-regulated DEGs and target genes (153 genes in up and 51 genes in down) were found in TCC and the least number of shared up-regulated DEGs and target genes (51 genes) were found in ccRCC, while the least number of shared down-regulated DEGs and target genes (35 genes) were found in TGCT (Figs. 2A-2C).

Network and GO analysis of shared genes involved between DEGs and target genes of DEmiRNAs in ccRCC,TCC and TGCT

Shared genes involved between DEGs and target genes of DEmiRNAs may play vital roles in the development process of each tumor, therefore we used these genes selected in ccRCC, TCC and TGCT for further analysis. Gene interaction scores were predicted in STRING (https://string-db.org/) and visualized utilizing Cytoscape software 3.4.0 (http://www.cytoscape.org/). GO analysis was conducted by the Blast2GO software (https://www.blast2go.com/).

In ccRCC, only up-regulated shared genes involved between DEGs and target genes of DEmiRNAs were found having strong interactions in STRING. Four gene interaction groups were found and ten significantly enriched biological processes of GO analysis were found, including negative regulation of gene expression, regulation of cAMP-mediated signaling, and fat pad development, etc. (Fig. 3).

In TCC, both up and down-regulated shared genes involved between DEGs and target genes of DEmiRNAs were found having strong interactions in STRING. In up-regulated interactions, four gene interaction groups were found and seven significantly enriched biological processes of GO analysis were found, including establishment or maintenance of cell polarity, protein localization to plasma membrane, calcium ion homeostasis, etc. (Fig. 4). In down-regulated interactions, one gene interaction groups were found and six significantly enriched biological processes of GO analysis were found, including small GTPase mediated signal transduction, muscle contraction, positive regulation of macrophage activation, etc. (Fig. 4).

In TGCT, only up-regulated shared genes involved between DEGs and target genes of DEmiRNAs were found having strong interactions in STRING. One gene interaction groups were found and three significantly enriched biological processes of GO analysis were found, including regulation of ion transmembrane transport, multicellular organism development and potassium ion import across plasma membrane (Fig. 5).

Investigating the survival data of selected genes

Survival data was downloaded in OncoLnc database (http://www.oncolnc.org/), as there were no data for three cancers we analyzed in the database, we used the data of kidney renal clear cell carcinoma (KIRC).

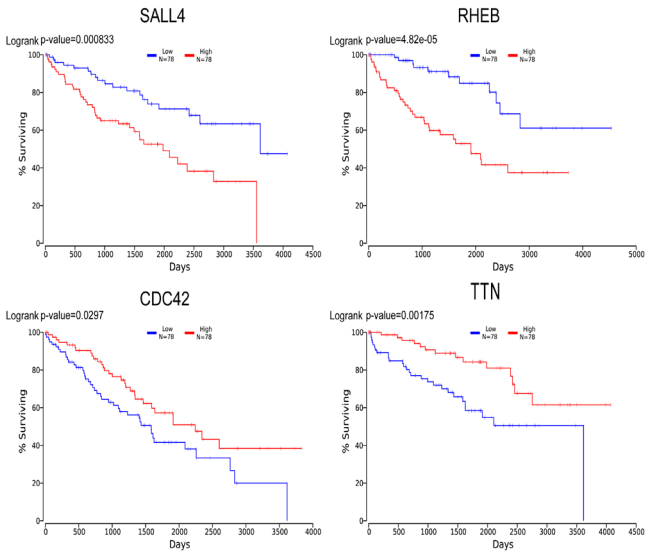


FIGURE 6. Survivalship curve of hub genes involved in the interaction network of three cancers. With the criteria of lower percentile 15/upper percentile 15 and logrank *p* value≤0.005, four genes affecting survivorship curve were selected, which were also in the hub interaction network analyzed in Figs. 3-5.

With the criteria of lower percentile 15/upper percentile 15 and logrank p value≤0.005, we selected four genes affecting survivorship curve, which were also in the hub interaction network analyzed in Figs. 3-5: *TTN* (up-regulated network in ccRCC), *CDC42* (up-regulated network in TCC), RHEB (down-regulated network in TCC) and *SALL4* (up-regulated network in TGCT). Lower expression of *SALL4* and *RHEB* were found beneficial for survival rate after over 3500 days' observation, while higher expression of *CDC42* and *TTN* were found beneficial for survival rate after over 3500 days' observation. These results validated that the genes we selected in the hub regulation network of three genitourinary cancers, also played important role in another genitourinary cancer (KIRC), which in turn proved the importance of utilizing the candidate genes in ccRCC, TCC and TGCT for future analysis.

Discussion

In this study, we utilized the original data (GSE31617) from GEO. Different from previous study, we focused on specific up and down-regulated DEGs and DEmiRNAs of each cancer and target genes of up and down-regulated miRNAs. We constructed gene interaction network of the shared genes of up or down-regulated DEGs and target genes of DEmiRNAs of each cancer. Moreover, in each cancer, we build regulatory networks of hub genes selected and conducted GO analysis of enriched genes. Furthermore, we chose four hub genes for

survival analysis in OncoLnc data base.

The largest number of DEGs were found in ccRCC (4592 DEGs), while the least number of DEGs were found in TGCT(3181 DEGs). However, the largest number of DEmiRNAs were found in TGCT (220 DEmiRNAs), while the least number of DEmiRNAs were found in ccRCC(105 DEmiRNAs). These results indicated that there may be no relationship between the number of DEGs and DEmiRNAs.

Similar findings were found after analyzing the DEGs and DEmiRNAs that were specifically expressed in TGCT, TCC and ccRCC. TGCT showed the least number of specific up-regulated DEGs and most abundant number of specific up-regulated DEmiRNAs, while ccRCC showed the most abundant number of specific up-regulated DEGs and least number of specific up-regulated DEmiRNAs. On the contrary, TGCT showed the most abundant number of specific down-regulated DEGs and least number of specific down-regulated DEmiRNAs, while ccRCC showed the least number of specific down-regulated DEGs and most abundant number of specific down-regulated DEmiRNAs.

We then combined DEGs with their target genes of DEmiRNAs, in both up and down-regulated comparisons. The largest number of shared up or down-regulated DEGs and target genes (153 genes in up and 51 genes in down) were found in TCC and the least number of shared up-regulated DEGs and target genes (51 genes) were found in ccRCC, while the least number of shared down-regulated DEGs and target genes (35 genes)were found in TGCT.

Shared genes involved between DEGs and target genes of DEmiRNAs may play crucial roles in the development process of each tumor, therefore we used these genes selected in three cancers for gene interaction network analysis.

In ccRCC, only up-regulated shared genes involved between DEGs and target genes of DEmiRNAs were found having strong interactions. Four gene interaction groups were found. Of the hub genes, *MBNL1* (muscleblind like splicing factor 1) is a RNA-binding protein (Delorimier *et al.*, 2017), and reduced cytoplasmic MBNL1 is an early event in a brain-specific mouse model of myotonic dystrophy (Wang *et al.*, 2017). *RASD2*(RASD family member 2) is a thyroid hormone target gene, which encodes for a GTP-binding protein enriched in the striatum where, among other functions, it modulates dopaminergic neurotransmission(Vitucci *et al.*, 2016). *DLG5* (discs large homolog 5) plays an important role in the maintenance of epithelial cell polarity, loss of *DLG5* promotes breast cancer malignancy by inhibiting the Hippo signaling pathway (Liu *et al.*, 2017). Though there were no functional reports of theses hub genes on ccRCC, these hub genes may play potential regulatory roles in ccRCC and our future study will focus on them.

In TCC, both up-regulated and down-regulated shared genes involved between DEGs and target genes of DEmiRNAs were found having strong interactions. In up-regulated interactions, four gene interaction groups were found. Of the hub genes, *KYNU* (kynureninase) is located on chromosome band 2q14-q23, where a linkage peak for essential hypertension was previously detected in the Chinese Han population (Zhang *et al.*, 2011). *DAPK1*(Death-associated protein kinase1) is an important tumor suppressor gene, which play roles in various tumors, including non-small cell lung cancer, cervical cancer, breast cancer, bladder cancer, etc. (Fernandez-Marcelo *et al.*, 2014; Xiong *et al.*, 2014; Loginov *et al.*, 2017; Xie *et al.*, 2017). In down-regulated interactions, one gene interaction groups were found. Of the hub genes, *ITGB3*(integrin beta 3) acts as a key regulator in reactive oxygen species-induced migration and invasion of colorectal cancer cells (Lei *et al.*, 2011), and it also plays roles in breast cancer and non-small-cell lung cancer (Bojesen *et al.*, 2005; Ni *et al.*, 2015). Though there were no functional reports of theses hub genes on TCC, these hub genes may play potential regulatory roles in TCC and our future study will focus on them.

In TGCT, only up-regulated shared genes involved between DEGs and target genes of DEmiRNAs were found having strong interactions. One gene interaction groups were found. Of the hub genes, *POU5F1*(also known as octamer-binding factor, *Oct-4* or *Oct-3*), is a novel prognostic marker after curative surgical resection in colorectal cancer (Miyoshi *et al.*, 2016), and it also plays roles in breast cancer, lung cancer and prostate cancer (Breyer *et al.*, 2014; Niu *et al.*, 2015; *Cai et al.*, 2016). *ZFP42* (zinc finger protein 42) has regulatory roles in in normal prostate epithelial cells and prostate cancer cells (Lee *et al.*, 2010). *DDX4* (the human ortholog of Drosophila Vasa) is an RNA helicase and is present in the germ lines of all metazoans tested, it functions in blood-derived cancer cell phenotypes and colocalizes with cancer stem cell marker CD133 in ovarian cancers (Kim *et al.*, 2014; Schudrowitz *et al.*, 2017). Though there were no functional reports of theses hub genes on TGCT, these hub genes may play potential regulatory roles in TGCT and our future study will focus on them.

To further validate the function of hub genes we selected, as there were no data for the cancers we studied, survival data of kidney renal clear cell carcinoma (KIRC), which is also one of genitourinary cancers were analyzed. We selected four genes affecting survivorship curve, which were also in the hub interaction network analyzed in three cancers: *TTN* (up-regulated network in ccRCC), *CDC42*(up-regulated network in TCC), *RHEB* (down-regulated network in TCC) and *SALL4*(up-regulated network in TGCT). *SALL4* (Sal-like protein 4) is recognized as a potential biomarker for assessing cancer prognosis, higher expression of *SALL4* predicts poor cancer prognosis in various cancers including gastric cancer, lung cancer, breast cancer and prostate cancer(Dirican *et al.*, 2016; Lai *et al.*, 2016; Yong *et al.*, 2016; Yuan *et al.*, 2016; Shen *et al.*, 2017). The small GTPase *RHEB* promotes cancer cell survival through p27Kip1-dependent activation of autophagy (Campos *et al.*, 2016), and it also functions in liver cancer, colon cancer cells, bladder cancer and prostate cancer(Kobayashi *et al.*, 2010; Campos *et al.*, 2013; Tigli *et al.*, 2013; Zheng *et al.*, 2015). *CDC42* (cell division control 42 homolog) has multiple functions in breast cancer, metastatic cancer, non-small cell lung cancer and colorectal cancer (Chrysanthou *et al.*, 2017; Humphries-Bickley *et al.*, 2017; Li *et al.*, 2017b; Valdes-Mora *et al.*, 2017). TTN (Topotecan) functions in ovarian cancer (Buckley *et al.*, 2005). In accordance with their functions in cancers, lower expression of *SALL4* and *RHEB* were found beneficial for survival rate after over 3500 days' observation, while higher expression of *CDC42* and *TTN* were found

beneficial for survival rate after over 3500 days' observation.

In summary, our results indicated that the genes we selected in the hub regulation network of three genitourinary cancers, also played important role in another genitourinary cancer, KIRC, which in turn proved the importance of utilizing the candidate genes in ccRCC, TCC and TGCT for exploring molecular targets, diagnostic biomarkers for the treatment of them.

Acknowledgments

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Author contributions

ML designed the experiments. XZ analyzed the data and wrote the paper. All the authors gave final approval of the version to be published, and declare no conflicts of interest.

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