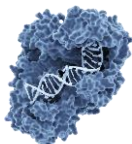

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Differential gene expression analysis of papillary thyroid carcinoma reveals important genes for lymph node metastasis

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Abstract: Background: Papillary thyroid carcinoma (PTC) is the most prevalent thyroid cancer. We explored the differential gene expression (DEG) of the transcriptional regulation in thyroid cancer with the main aim of determining the genes involved in lymph node metastasis (LNM) in PTC. Methods: We employed a bioinformatics pipeline for RNA-Seq analysis in PTC with and without LNM at the gene expression level. We performed read mapping, read quantitation, and DEG analysis using STAR, Cufflinks, and Cuffdiff, respectively. Subsequently, functional annotation and pathway enrichment were carried out using FunRich (functional enrichment analysis tool). Results: Expression profiling revealed changes in the PTC with LNM (33 genes at p-value < 0.05 and $\log_2$ fold change |1.0|)

compared to the adjacent normal thyroid, whereas 69 genes showed differential expressions in the PTC with lymph node negative (LNN) versus adjacent normal thyroid. We identified 31 significant DEGs in PTC LNM versus PTC LNN; and 44 significant DEGs between adjacent normal thyroid tissues from PTC LNM and PTC LNN. The biological processes of genes expressed at higher levels in PTC LNM compared to PTC LNN were involved in cell communication, energy pathway, and metabolism, whereas ion transport, energy pathways, and regulation of nucleobase, nucleoside, nucleotide, and nucleic acid metabolisms, were downregulated. Conclusion: These findings provide further evidence for the role of cellular transport regulatory processes in metastatic disease.

Keywords: Papillary thyroid carcinoma; lymph node metastasis; lymph node negative; differentially expressed genes

1. Introduction

The number of new cases of thyroid cancer including papillary thyroid carcinoma (PTC) is steadily increasing over the past decade especially in Asian countries compared to European countries[1]. Thyroid cancer has a high survival rate because of early detection through screening and advances in medical treatment[2,3]. There are four types of thyroid cancer namely follicular (FTC), medullary (MTC), anaplastic (ATC) and papillary (PTC), and the latter is the most common, contributing 80% to 85% of all thyroid cancers[4,5]. The survival rate for PTC is high (98%) in Europe and North America due to improved diagnosis, management, and treatment of the disease[6]. Yet, a subset of PTC patients displayed aggressive features associated with poor prognosis, and one of the features is the presence of lymph node metastasis (LNM)[7].

Several studies have shown the different molecular landscapes of LNM in various types of cancer, including oral, pancreatic, colorectal, and breast cancer[8]. However, the gene expression profile in LNM of PTC has not been extensively done, with only a handful of published findings[9]. Understanding the molecular and cellular mechanisms of LNM in PTC is required to improve prognostication.

In this study, we investigated the gene expression profiles of PTC with and without LNM and conducted a comprehensive analysis of gene expression of thyroid tissue samples via RNA-Seq. The differentially expressed genes (DEGs) identified in PTC with and without LNM might be the potential biomarkers and provide the basis for further exploration of the mechanisms of metastatic disease in PTC.

2. Materials and methods

2.1. Ethics statement and sample collection

This study was conducted according to the Universiti Kebangsaan Malaysia Research Ethics Committee (UKMREC) (reference: UKM 1.5.3.5/244/UMBI-2015-002). Ten fresh-

frozen tumour PTC tissues specimens from the cancer Biobank were subjected to cryosectioning and stained with haematoxylin and eosin (H&E). The pathologist reviewed the percentage of tumour cells and normal cells in the slides. Tumour tissues containing more than 80% of cancer cells and normal tissues with less than 20% necrosis were subjected to RNA extraction.

2.2. Total RNA isolation

According to the manufacturer's protocol, total RNA was isolated from the samples using AllPrep DNA/RNA/miRNA Isolation Kit (Qiagen, Germany). The total RNA quality and quantity were carried out using an ND-1000 spectrophotometer (Thermo Scientific, USA) and Qubit™ fluorometer (Invitrogen, USA). Before library preparation, the RNA quality was measured using an Eukaryote Total RNA Nano chip on Bioanalyzer 2100 (Agilent Technologies, USA).

2.3. Library preparation and RNA sequencing

Libraries were constructed using the Ion Total RNA-Seq v2 kit (Life Technologies, USA) according to the manufacturer's protocol. Briefly, 2 µg of total RNA was subjected to ribosomal removal using RiboMinus Eukaryote kit (Life Technologies, USA). ERCC RNA Spike-In Control Mixes (Thermo Scientific, USA) was added into rRNA depleted-RNA before RNA fragmentation using RNase III. The fragmented RNA was quantified using Qubit™ fluorometer (Invitrogen, USA) and Agilent RNA 6000 Nano Kit (Agilent Technologies, USA), followed by hybridization and ligation to adaptors, reverse-transcribed, purified, size-selected, and amplified. The final library concentration was assessed using Agilent High Sensitivity DNA chip (Agilent Technologies, USA) and normalized from 100 pM to 130 pM for clonal amplification. Clonal amplification was performed on Ion Chef System using Ion PI IC 200 kit followed by sequencing using PI BC v2 chip on Ion Proton system (all from Life Technologies, USA).

2.4. Read mapping and gene quantification

Raw reads (FASTQ files) were processed using AfterQC[10] to clean the data and obtain high-quality reads by removing reads that contain adapter or poly-N and low quality reads. GC content of the clean data was then calculated. All downstream analyses were based on clean data with high quality. Sequencing reads were mapped with STAR (v2.0.9[11,12] to the human genome sequence assembly (GRCh38.89). The mapped reads of each sample were assembled using Cufflinks[13].

2.5. Differential gene expression

Differential gene expression (DEG) analysis was performed using Cuffdiff[13]. Four comparisons were performed: PTC LNM (lymph node metastasis) versus PTC LNN (lymph node negative), PTC LNM versus normal thyroid, PTC LNN versus normal thyroid, and normal thyroid from PTC LNM patients versus normal thyroid from PTC LNN. Genes with

a p-value of less than 0.05 and showing a $\log_2$ fold change |1| were considered as significantly differentially expressed.

2.6. Gene Ontology and KEGG pathway enrichment analysis

Next, we performed a Gene Ontology (GO) analysis of these DEGs. Functional enrichment analysis focusing on biological processes was performed on datasets by using FunRich software[14]. Settings were set at a default parameter, and fold enrichment with unadjusted p-values were computed using a modification of the Fisher's exact test. We used KEGG, a database resource for understanding high-level functions and utilities of the biological system, to test the statistical enrichment of differential expression genes in KEGG pathways[15].

3. Results

3.1. Data characterization of sequencing and mapping

Our RNA-Seq yielded 22.2 to 80.3 million reads. Using STAR aligner, the proportion of reads that were successfully mapped to the Ensembl reference genes ranged from 77% to 85%, indicating that the data produced are of high quality. Gene expression levels were estimated using Cufflinks plotted in Figure 1 with Pearson correlation coefficients of the estimated abundances between the two tissues. It showed that the gene quantification with Cufflinks was consistent across different comparisons.

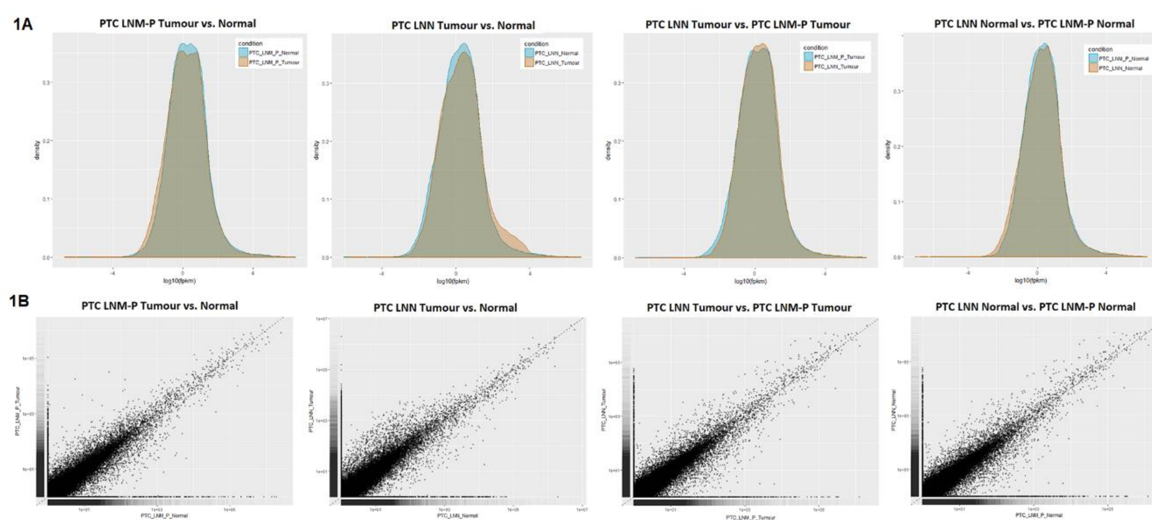

Figure 1. Differential gene expression analysis of PTC and normal thyroid tissue. (A) Density plots showing the expression level distribution for all genes in the two tissues. FPKM = fragments per kb of transcript per million fragments mapped. (1B) The scatter plot of global expression for each comparison between samples where the Pearson correlation coefficient (PCC) is shown. Each dot represents one gene that has detectable expression in either tissue.

3.2. Differential gene expression

We identified 33, 69, 31, and 44 significant DEGs between the PTC LNM versus normal, PTC LNN versus normal, PTC LNM versus PTC LNN, and normal thyroid from PTC LNM versus normal thyroid from PTC LNN, respectively (Table 1). The complete lists of the DEGs were provided in Supplementary Table S1. Up- and down-regulated genes comparing PTC LNM versus PTC LNN were shown in Figure 2. In the group comparison of PTC LNM versus normal thyroid, up-regulated transport-related genes in the PTC LNM included *GABRE* and *NUP160*, while down-regulated genes were *RYR3* and *HBA2*. In the PTC LNN versus normal thyroid, the upregulated genes included *LCN2*, *RYR3*, *KCNQ3*, *GRIA3*, *SLC13A5*, and *SLC34A2* but downregulated transport-related genes were not found. While in the group comparison of PTC LNM versus PTC LNN, there were two up-regulated transport-related genes, *SLC13A5* and *SYBU*, and three down-regulated transport-related genes, which were *GRID1*, *SCN8A*, and *TRPM2*. In the normal thyroid from both PTC LNM and PTC LNN, 17 up-regulated and 27 down-regulated with only two transport-related genes were being differentially expressed. The down-regulated transport-related genes were *RYR3* and *TMEM199*. The overlap of the DEGs among the four different groups was shown in a Venn diagram in Figure 3.

Table 1. Significant differently expressed genes (p-value < 0.05 and $\log_2$ fold change |1|) in PTC LNM versus PTC LNN, normal thyroid (PTC LNM) versus normal thyroid (PTC LNN), PTC LNM versus normal, and PTC LNN versus normal group.

Group	PTC LNM versus normal thyroid	PTC LNN versus normal thyroid	PTC LNM versus PTC LNN	Normal thyroid (PTC LNM) versus normal thyroid (PTC LNN)
Up-regulated gene	14	50	14	17
Down-regulated gene	19	19	17	27
TOTAL	33	69	31	44

LNM: lymph node metastasis; LNN: lymph node negative; PTC: papillary thyroid cancer

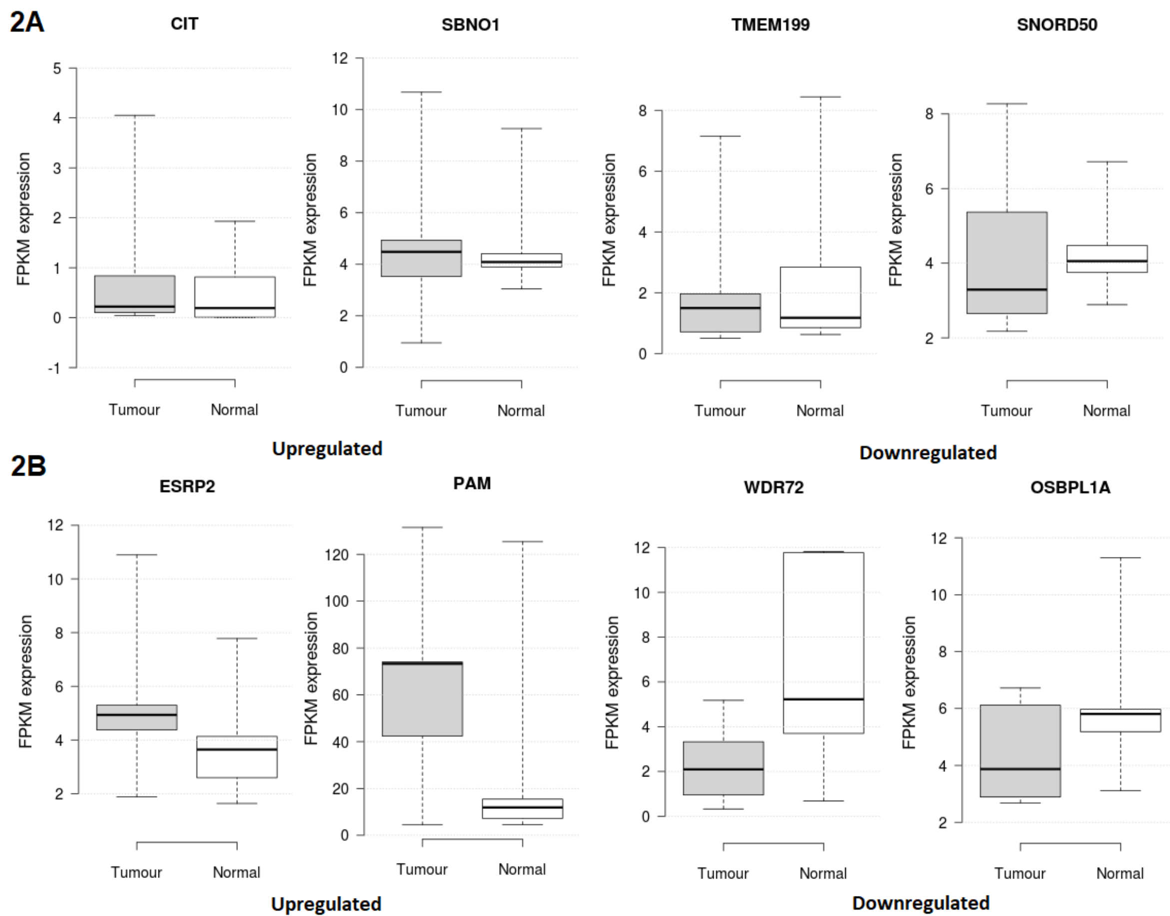

Figure 2. Expression level of five up- and down-regulated in PTC LNM versus PTC LNN. (2A) Boxplots illustrated FPKM expression value in PTC LNM versus PTC LNN (*SLC13A5* and *SYBU*) and (2B) boxplots demonstrated FPKM expression value in PTC LNM versus PTC LNN (*GRID1*, *SCN8A*, and *TRPM2*).

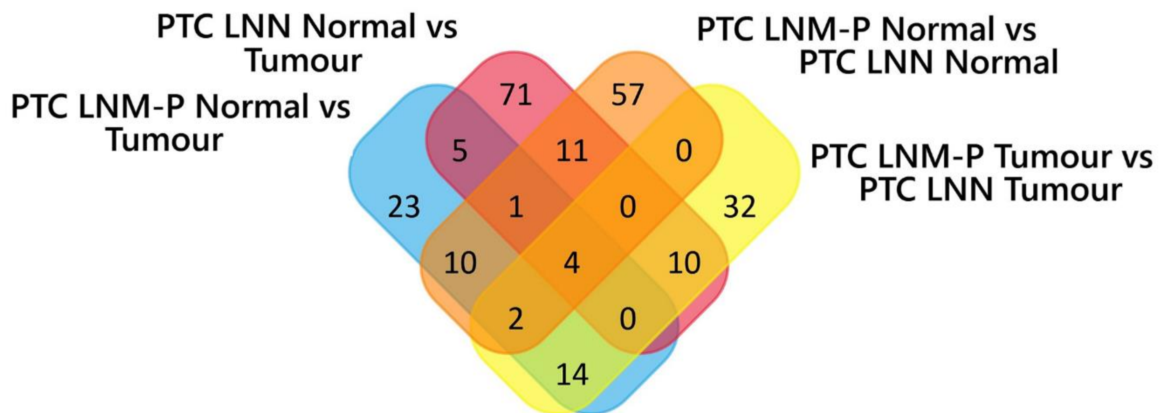

Figure 3. Venn diagram representing differentially expressed transport-related genes overlapping between the samples. The Gene list from each group comparison showed the frequency of transport-related genes.

3.3. Enriched functions of differentially expressed gene

The functional analysis showed that the primary biological processes altered in PTC LNM versus normal thyroid were protein metabolism, metabolism, energy pathways, cell growth and/or maintenance, and transport. These processes are frequently involved in cancer. To improve our understanding of the function of the DEGs, we performed an enrichment analysis of Gene Ontology (GO) for the up-and down-regulated genes, as illustrated in Figure 4. First, we performed enrichment tests to detect the functional categories for significantly up- and down-regulated genes detected by Cuffdiff in the PTC LNM, PTC LNN, and adjacent normal tissue using the FunRich software. The GO categories that were greatly enriched in the regulated genes from PTC LNM versus normal thyroid, PTC LNN versus normal thyroid and PTC LNM versus PTC LNN were selected. The up- and down-regulated genes in all comparisons in PTC were categorized into 177 functional categories. We identified 14 up- and 19 down-regulated genes in PTC LNM versus normal thyroid and 50 up-regulated and 19 down-regulated genes in PTC LNN versus normal thyroid. For instance, the biological processes for up-regulated genes, which include “protein targeting”, “inflammatory response”, “regulation of cell growth”, “cell differentiation” and “transport” are essential to cancer progression[16,17]. Whilst the upregulated genes in PTC LNN versus normal included “cell growth and maintenance”, “energy pathway”, “pyrimidine salvage”, “transport”, “ion transport” and “protein metabolism”. On the contrary, genes that were downregulated in PTC LNN versus normal thyroid included those involved in “cell communication”, “cell differentiation”, “cell proliferation”, “steroid hormone receptor signaling pathway”, and “vesicle-mediated transport” and “apoptosis”. Interestingly, the biological process of “transport” appeared in most of the group comparisons suggesting that transport genes could potentially govern the metastasis cancer progression in thyroid cancer.

Detailed analysis of functional annotation was performed using FunRich to analyze which KEGG pathways were enriched with PTC-specific down-regulated genes. The pathways enriched with DEGs are listed in Table 2. RHO GTPases activate CIT interaction pathway was affected in two out of four pairwise comparisons (PTC LNM versus normal thyroid and PTC LNM versus PTC LNN, and other gene regulation alterations in the extracellular matrix (ECM) organization pathway were involved in cancer tissue. Furthermore, the ChREBP activates metabolic gene expression pathway was enriched in the DEGs identified from the PTC tissue.

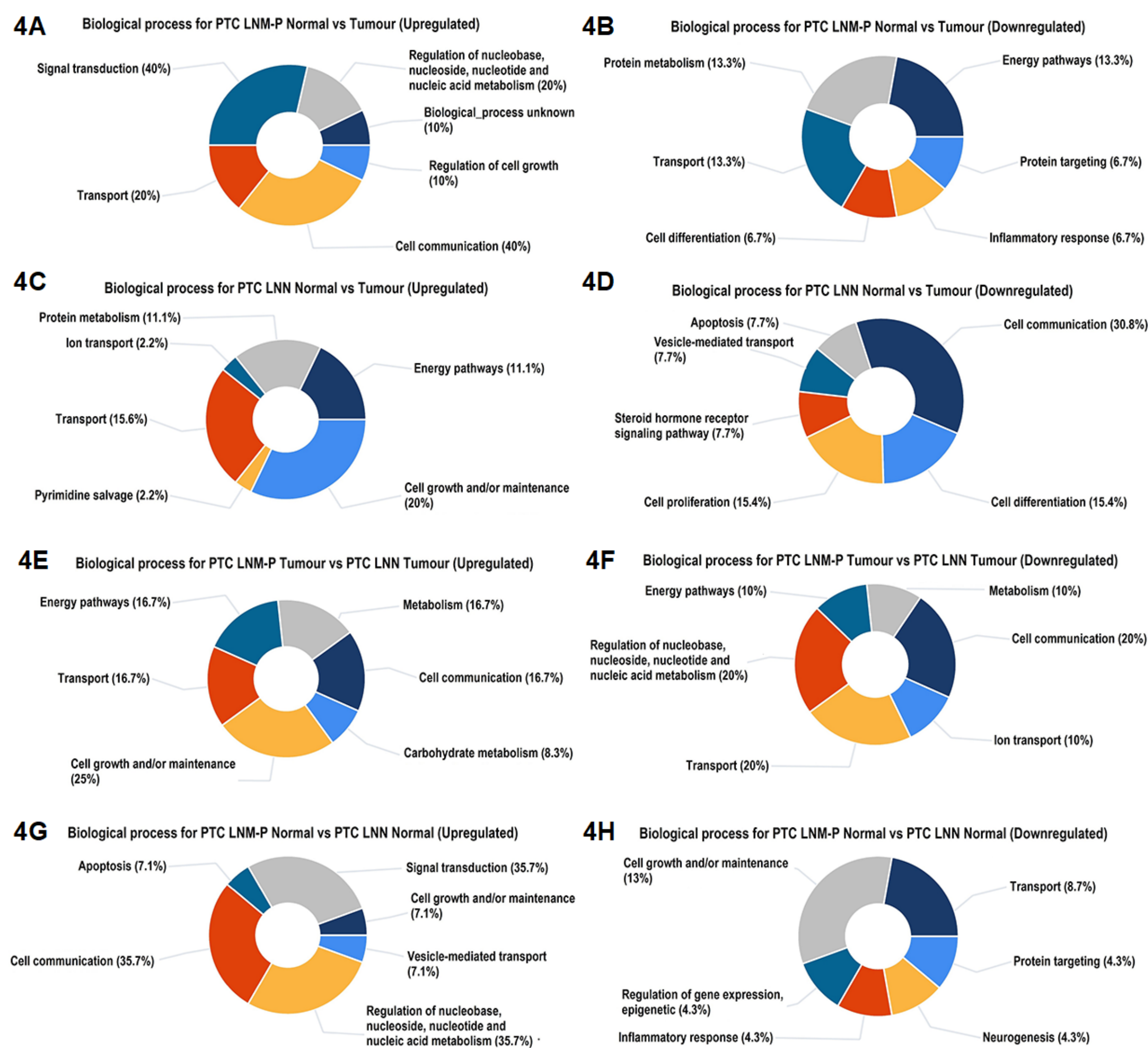

Figure 4. Gene enrichment biological processes across the different groups.

Table 2. KEGG pathway of enriched differentially expressed genes.

Comparison	Pathway ID	Pathway Name	p-value	FDR
PTC LNM versus normal thyroid	1270056	Acyl chain remodeling of DAG and TAG	1.272E-4	2.480E-2
	1269514	RHO GTPases activate CIT	4.759E-4	4.640E-2
PTC LNN versus normal thyroid	1270114	ChREBP activates metabolic gene expression	6.05E-04	2.62E-01
	1270254	Non-integrin membrane-ECM interactions	9.19E-04	3.99E-01
	1470923	Interleukin-4 and 13 signaling	1.29E-03	5.59E-01
	1270244	Extracellular matrix organization	1.44E-03	6.26E-01
PTC LNM versus PTC LNN	1269514	RHO GTPases activate CIT	5.60E-04	5.93E-02
	1457800	MET activates PTK2 signaling	7.79E-04	8.25E-02
	1108786	mucin core 1 and core 2 O-glycosylation	1.22E-03	1.30E-01
	1270253	Laminin interactions	1.22E-03	1.30E-01
	1383022	FGFR2 alternative splicing	1.53E-03	1.63E-01
PTC LNM (normal) versus PTC LNN (normal)	852705	MicroRNAs in cancer	2.38E-05	7.58E-03
	142173	Serine biosynthesis (phosphorylated route)	2.98E-03	9.48E-01

CIT: citron rho-interacting serine/threonine kinase; ChREBP: carbohydrate response element binding protein; DAG: diacylglycerol; ECM: extracellular matrix; LNM: lymph node metastasis; LNN: lymph node negative; MET: MNNG HOS transforming gene; PTC: papillary thyroid cancer; PTK2: protein tyrosine kinase; TAG: triacylglycerol

GO analysis was further refined to identify which DEGs were involved in transport, which was an important component of metastasis in PTC and illustrated in Table 3. For *in silico* validation, the expression signatures of selected thyroid-specific genes (*GRID1*, *KCNQ3*, and *SLC34A*) were retrieved from the RNA-Seq data of primary tumors in The Cancer Genome Atlas (TCGA) portal (Figure 5).

Table 3. Transport-related genes expression in RNA-Seq data of thyroid cancer.

Group	PTC LNM versus normal thyroid	PTC LNN versus normal thyroid	PTC LNM versus PTC LNN	Normal thyroid (PTC LNM) versus normal thyroid (PTC LNN)
Up-regulated gene	*GABRE, NUP160*	*LCN2, RYR3, SLC34A2, KCNQ3, SLC13A5, GRIA3*	*SLC13A5, SYBU*	-
Down-regulated gene	*RYR3, HBA2*	-	*GRID1, SCN8A, TRPM2*	*RYR3, TMEM199*

GABRE: gamma-aminobutyric acid type A receptor subunit Epsilon; GRIA3: glutamate ionotropic receptor AMPA type subunit 3; GRID1: glutamate receptor delta-1 subunit; HBA2: haemoglobin alpha 2; KCNQ3: potassium voltage-gated channel subfamily Q member 3; LCN2: lipocalin 2; LNM: lymph node metastasis; LNN: lymph node negative; NUP160: nucleoporin 160; PTC: papillary thyroid cancer; RYR3: ryanodine receptor 3; SCN8A: sodium voltage-gated channel alpha subunit 8; SLC13A5: solute carrier family 13 member 5; SLC34A2: solute carrier family 34 member 2; SYBU: syntabulin; TMEM199: transmembrane protein 199; TRPM2: transient receptor potential cation channel, subfamily M, member 2

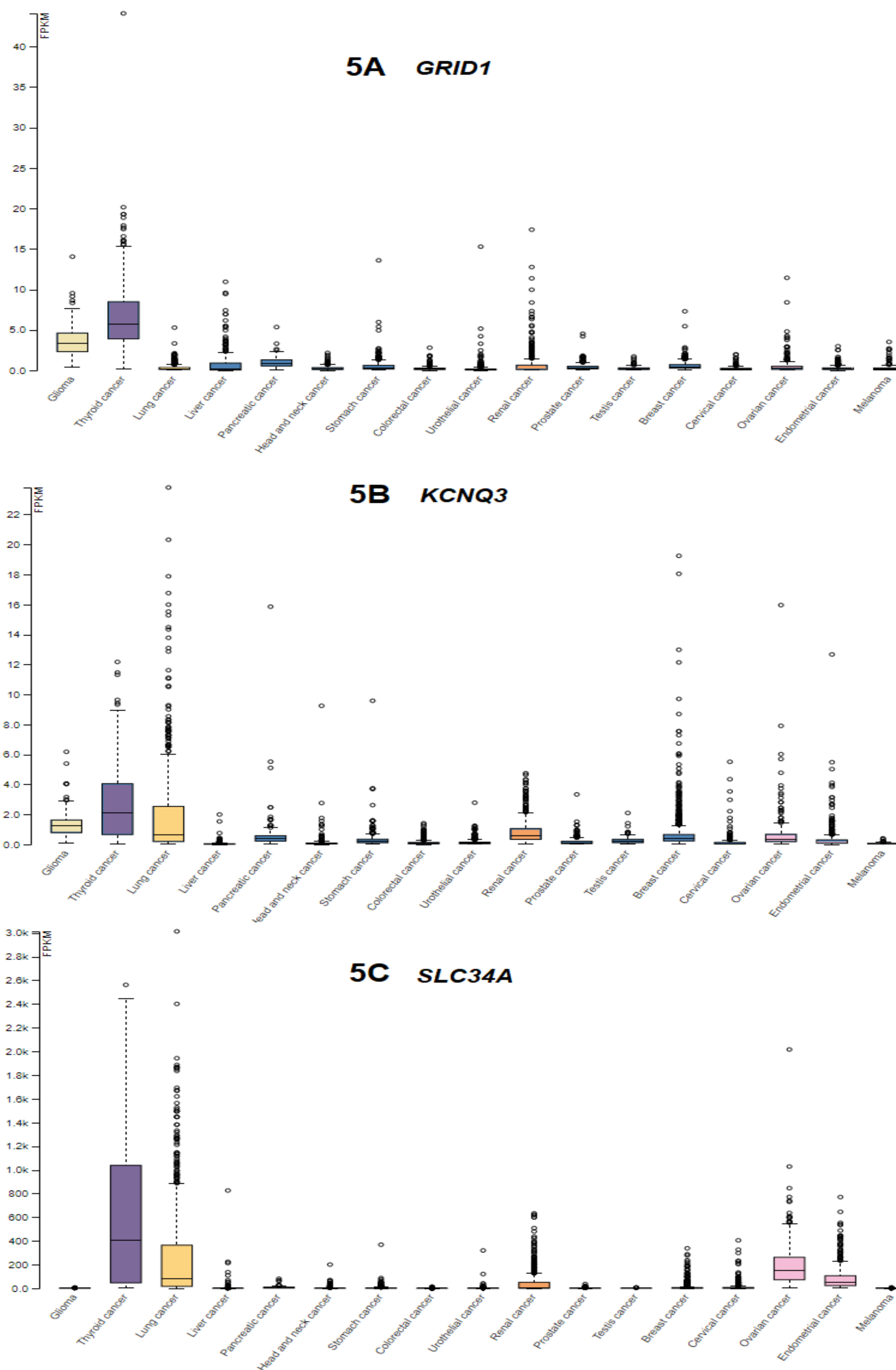

Figure 5. Gene expression overview of TCGA data; higher in thyroid cancer. (5A) *GRID1* (5B) *SLC34A2* and (5C) *KCNQ3*.

4. Discussion

Our study aimed to identify genes responsible for lymph node metastasis in PTC. We profiled the whole transcriptome of PTC LNM, PTC LNN, and normal thyroid tissues using ribosomal-reduced RNA-Seq. Research on thyroid cancer progression has been well-studied using microarray and RNA-Seq. We identified several transporter-related genes associated with PTC LNM, PTC LNN, and adjacent normal thyroid. Ion transport process happens during the multi-stage cancer progression, from a normal cell to cancer cell and finally to metastasis. Our results suggested an important role of transporter genes during lymph node metastasis in thyroid cancer.

We identified 177 DEGs within the various comparison groups. However, a KEGG pathway enrichment analysis showed that the regulated genes were significantly enriched in the RHO GTPases activated CIT pathway in two group comparisons: PTC LNM versus PTC LNN and PTC LNM versus normal thyroid. This pathway is closely related to cancer progression[18,19]. For instance, aberrant activity of Rho small G-proteins, particularly Rac1 and their regulators, is a hallmark of cancer and contributes to the tumorigenic and metastatic phenotypes of cancer cells[20]. Two up-regulated transporter genes (*GABRE* and *NUP160*) and two down-regulated genes (*RYR3* and *HBA2*) were identified in PTC LNM versus normal thyroid. The *GABRE* gene encodes for the gamma-aminobutyric acid type A receptor epsilon subunit, which is a multisubunit chloride channel that facilitates the synaptic transmission in the central nervous system (CNS). The miR-224/452 cluster is co-expressed with its host gene *GABRE* located in the intron of GABRE gene coding for gamma-aminobutyric acid (GABA) receptor[21], and its expression is directly activated by transcription factor E2F1 through transactivation of the *GABRE* gene[22]. This gene is up-regulated in medulloblastomas, lung cancer, bladder cancer, prostate cancer, and colorectal cancer[23–26]. Many studies reveal that miRNAs are involved in carcinogenesis, and miRNA expression has been found in many types of cancer[27–29].

In contrast, the *GABRE*~miR-452~miR-224 locus has been reported to be down-regulated and hypermethylated in prostate cancer, suggesting its potential as a new epigenetic candidate biomarker for prostate cancer diagnosis and prognosis[24,30]. It has also been up-regulated in hepatocellular carcinoma[31]. MiR-224 is also up-regulated in PTC, MTC, FTC and ATC[32]. Many studies on different types of cancer suggest that miR-224 promotes tumorigenesis in colorectal cancer[33,34], non-small cell lung cancer[35], medullary thyroid carcinoma[36] and hepatocellular carcinoma[37]. In contrast, some studies show that it suppresses tumorigenesis in prostate cancer[24,30], and breast cancer[38,39]. *GABRB2*, which is part of the GABA receptors gene family, plays a crucial role in the lymph node metastasis of PTC[40]. Its function is closely related to the nervous system, but its role in cancer remains uncertain[41].

We identified two down-regulated genes in PTC LNM versus normal thyroid, namely, *NUP160* and *RYR3*. The *NUP160 gene* encodes the nuclear pore complex protein Nup160, which mediates molecule transport across the nuclear envelope[42] and is thought to be

involved in cancer progression[43]. Up-regulation of this gene may indicate its role in lymph node metastasis. One study reported a novel frequent fusion gene, *NUP160-SLC43A3*, in angiosarcoma patients and deduced that this fusion protein might cause rapid tumour progression[44]. This novel fusion could also provide a possible therapeutic target for many human malignancies[45,46]. Our findings suggest an oncogenic role of *GABRE* and *NUP160* in thyroid carcinogenesis.

The *RYR3* gene encodes a ryanodine receptor, which releases calcium from intracellular storage for use in many cellular processes[47,48]. It is imperative for the growth, morphology, and migration of breast cancer cells, and studies have shown that it is commonly expressed in breast cancer[49,50]. Functional ryanodine receptor is also involved in the apoptosis of *in vitro* human prostate cancer LNCaP cells[51]. This gene's Gene Ontology (GO) analysis included calcium ion binding and calmodulin binding. Additionally, this gene is also involved in transport, suggesting that it might be involved in the process of metastasis in PTC.

In comparing tumour samples of PTC LNM and PTC LNN, we identified two up-regulated transport-related genes (*SLC13A5* and *SYBU*) and three down-regulated transport-related genes (*GRID1, SCN8A,* and *TRPM2*). The *SLC13A5* (solute carrier family 13 members 5) gene encodes for proteins that transport citrate from the cytoplasm into cells and is mostly expressed in liver cancer[52] via regulation of metabolic processes[53]. It is a sodium-coupled transporter that facilitates the cellular uptake of citrate and plays vital role in synthesizing fatty acids and cholesterol[52].

Syntabulin-kinesin-1 family member 5B (*SYBU*), a subset of the kinesin motor-adaptor complex is important for axonal transport and is involved in neuronal development [54]. It encodes syntabulin, a microtubule-related protein that facilitates the transport of vesicles to neuronal processes[55]. Functional analysis of *SYBU* revealed its involvement in various binding function such as protein binding, microtubule binding, syntaxin-1 binding, and kinesin binding[56]. It has been reported to be over-expressed in pancreatic adenocarcinoma, breast cancer and bladder cancer[57]. Thus far, no study has been conducted on this gene involvement in thyroid cancer.

In our study, three glutamate receptor subunits: *GRID1* (ionotropic glutamate receptor), *SCN8A* (sodium voltage-gated channel) and *TRPM2* (transient receptor potential cation channel) were significantly up-regulated in PTC LNM tissues. Ion transport is believed to be involved in cancer progression, starting with transforming a normal cell into a cancer cell and until the metastasis stage[58]. Moreover, this derangement in the ion transport mechanism is one of the hallmarks of cancer and could be a key event in tumour metastasis [59]. The study by Stepulak et al. also suggested glutamate as a potential growth factor in tumorigenesis [60]. Glutamate receptor subunits: *NR1-NR3B*, *GluR1-GluR7*, *KA1*, *KA2,* and *mGluR1-mGluR8*, have been shown to be differentially expressed in multiple cancer cell lines (TE671, SK-NA-S, FTC 238, SK-LU-1, MOGGCCM, RPMI 8226, A549, HT 29, Jurkat E6.1, T47D, LS180, U87-MG, and U343)[61,62] and tumours including thyroid cancer, hepatocellular carcinoma

(*GRIK1*), colorectal cancer (*mGluR4*)[63], melanoma (mGluR1)[64], and serous ovarian adenocarcinoma (*GRIA2*)[65]. The *GRID1 gene* showed increased expression in colorectal cancer and breast cancer[66], and down-regulated expression in non-small cell lung cancer of node-positive cases[67]. Thereby, *GRID1* might play an essential role in the progression of PTC. It could also be used as a prognostic biomarker and a therapeutic target[68].

Another gene up-regulated in PTC LNM is *SCN8A* which encodes a protein of the ion pore region of the voltage-gated sodium channel (VGSC) and is crucial for the rapid membrane depolarization[69]. This gene is over-expressed in cervical cancer and prostate cancer[70]. Additionally, the expression of VGSC is associated with cancer cell migration, invasion, and metastasis[71]. Inhibition of VGSC subunits has been suggested as a suitable treatment strategy to decrease the metastatic spread of prostate cancer[70]. A detailed review of VGSC by Wang et al. indicated that upregulation of *SCN8A* gene might increase cancer cell growth and promote metastasis. They also suggested that inhibiting sodium channels in both immune and cancer cells might reduce metastases[37].

Based on our results, the *TRPM2* gene, which encodes for the non-selective calcium-permeable cation channel, was up-regulated in PTC LNM. Our study showed concordance with other studies where *TRPM2* was over-expressed in many cancers, including but not limited to bladder cancer[72], gastric cancer[73(p2)], lung cancer, and breast cancer[74]. In 2013, Liu and the coauthors reported activation of *TRPM2* gene by irradiation via PARP1 activation. They contributed to the irreversible loss of salivary gland function, suggesting that the disordered expression of TRPM2 might be associated with the occurrence of head squamous cell carcinoma (HSCC)[75(p2)]. In addition, a group of researchers from China proposed that *TRPM2* was essential in the regulation of migration and survival of SCC cancer cells. At the same time, another study described that TRP channels were clinically valuable for cancer diagnosis and prognosis[76]. Our study identified a prominent association between *GRID1*, *SCN8A*, and *TRPM2* with LNM in PTC. These findings suggest that the upregulation of *GRID1* and *SCN8A* is related to PTC metastasis. Nevertheless, further studies are required to explore the potential of *GRID1* and *SCN8A* as molecular biomarkers of PTC.

Three thyroid-specific gene expression signatures (*GRID1, KCNQ3,* and *SLC34A*) were up-regulated in thyroid cancer based on the RNA-Seq data of primary tumours in The Cancer Genome Atlas (TCGA) portal, and this observation was concordant with our results. In our study, the KEGG pathway and GO enrichment analysis showed many transport pathways involved in lymph node metastasis. Future studies could focus on these transporter genes and its related pathways in cancer, such as the glucose and hexose pathways, which had been discussed previously by Adekola et al.[77].

The functional analyses of genes differentially enriched in PTC LNM and PTC LNN further supported the hypothesis that lymph node metastasis was associated with specific essential genes and pathways. The results from our study showed the similarities found in terms of the role of crucial transport pathways, especially from the perspective of metastasis in PTC. We screened the DEGs linked to the transport-related pathways to PTC by using the

TCGA database to confirm our findings. Among the 17 transport-related DEGs, only four genes (*GABRE, HBA2, KCNQ3,* and *SLC34A2*) showed similar expressions to our conclusions. Another 13 genes required further validation to confirm the results.

5. Conclusion

In conclusion, we have identified key mRNA signatures of PTC LNM and PTC LNN. The DEGs might be promising biomarkers of PTC LNM and could provide a basis for further exploration of the tumorigenesis PTC. These genes may play critical roles in metastasis and survival in PTC patients. These results offer new insights on metastasis in PTC and eventually lead to better diagnosis and possibly new therapeutic targets for thyroid cancer.

Author Contributions: N-SAM conceived the idea, supervised the project, and applied for the grant that funded the work. AMY and SS performed laboratory experiments. WFWMN performed the bioinformatics analyses and generated the tables and figures. RM, SNAS and NHML are the thyroid surgeons involved in specimen collection, and IMR is the pathologist who confirms the diagnosis. WFWMN, N-SAM, FYFT, NFMP and L-HL wrote and edited the manuscript.

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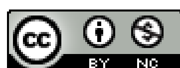
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