Transcriptomic profiling of parathyroid tumors reveals distinctive molecular characteristics of carcinoma and adenoma

Se-Young Jo[1*], Namki Hong[2*], Seunghyun Lee[2], Jong Ju Jeong[3], Jeongsoo Won[1], Jiho Park[1], Kijung Kim[4], Sang Kyum Kim[4], Sangwoo Kim[1**], Yumie Rhee[2**]

[1]Department of Biomedical Systems Informatics and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea
[2]Department of Internal Medicine, Severance Hospital, Endocrine Research Institute, Yonsei University College of Medicine, Seoul, Korea
[3]Department of Surgery, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea
[4]Department of Pathology, Yonsei University College of Medicine, Seoul, Korea
*These authors contributed equally
**Co-corresponding authors

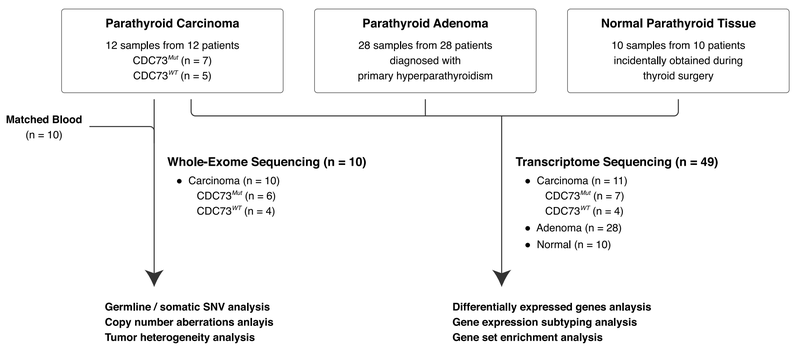
TGIL
YONSEI UNIVERSITY
TRANSLATIONAL GENOME INFORMATICS LABORATORY

ABSTRACT

Parathyroid carcinoma is a rare malignancy which remains as a clinical unmet need lacking effective therapeutic intervention. In this study, we compared mutational profile of parathyroid carcinoma, adenoma, and normal parathyroid tissue using RNA-Seq based transcriptomics analysis and whole-exome sequencing. A total of 50 parathyroid specimens [parathyroid carcinoma (n=12), adenoma (n=28), and normal tissue incidentally obtained from thyroidectomy for various reasons (n=10)] from 50 individuals (women n=41, 80%) were analyzed. CDC73 mutation was found in 7 of 12 carcinoma specimens, which harbored germline mutation in 6 of them.

Transcriptional profiling revealed 647 carcinoma-specific differentially expressed genes (DEGs) compared to adenoma and normal tissues. Hierarchical clustering with carcinoma-specific DEGs detected two distinctive clusters (carcinoma clusters vs. normal and adenoma clusters). Carcinoma-specific DEGs include upregulation of WT1, SLC17A8, ANGPTL4, PRUNE2, MYC and downregulation of PIK3C2G. Carcinoma-specific DEGs were associated with MYC targets, G2M check point, and epithelial mesenchymal transition pathway by gene set enrichment analysis. Among carcinomas, CDC73Mut and CDC73WT differ by 393 DEGs, which revealed association of CDC73Mut with MYC targets whereas CDC73WT was associated with epithelial mesenchymal transition. (Immunohistochemistry staining of FFPE samples revealed relatively high WT1 expression in carcinoma compared to adenoma and normal tissues.) In summary, parathyroid carcinoma had distinctive transcriptional profiles compared to normal parathyroid tissue and parathyroid adenoma, which might provide additional diagnostic value.

METHODS

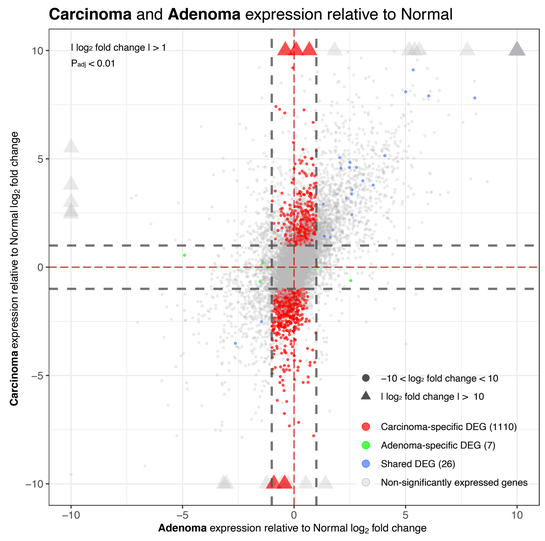


50 parathyroid specimens (28 adenomas, 12 carcinomas, and 10 normal parathyroid tissues) from 50 individuals (women n=41, mean age 51 years) through 2015-2019, Severance hospital, Seoul, Republic of Korea were collected for this study. All adenoma and carcinoma specimens were obtained from FFPE samples archived after surgery, and normal parathyroid tissues were incidentally obtained from thyroidectomy for benign thyroid diseases or non-metastatic thyroid cancers.

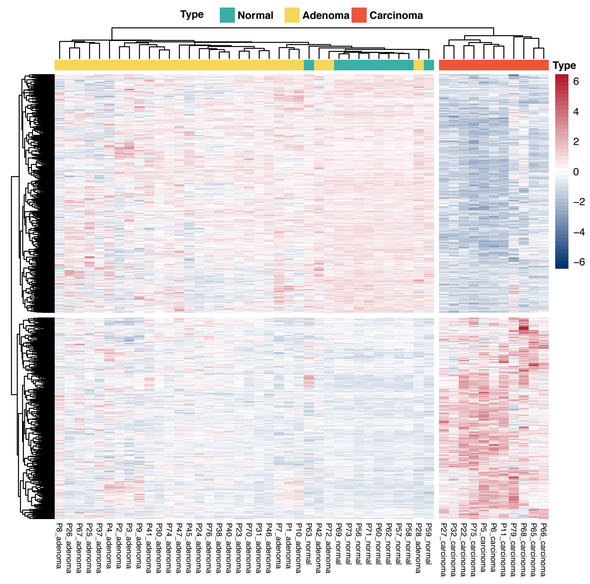
Transcriptome sequencing was performed on 49 specimens consisting of 28 adenomas, 12 carcinomas, and 10 normal parathyroid tissues, and whole-exome sequencing was performed for 10 carcinoma samples (200x) for accurate genomic analysis, and a total of 20 WES data were generated by adding matched blood samples obtained with the consent of the patient.

RESULTS

Identification of differentially expressed genes of parathyroid carcinoma



By performing 2-axis DEG analysis on the 49 transcriptomic sequencing data (28 adenomas, 12 carcinomas, and 10 normal parathyroid) collected and produced for this study, 1,110 carcinoma-specific DEGs and 7 adenoma-specific DEGs were obtained. (padj < 0.01 & log2 fold change > 1, red and green dots) In addition, we were able to find 25 shared DEGs that could not be found in conventional 1-axis DEG analysis which comparing carcinoma versus adenoma directly. (Blue dots) The adenoma-specific DEGs were found in a relatively small number compared to the number of carcinoma-specific DEG.

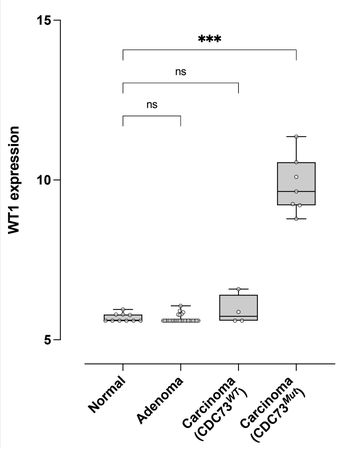


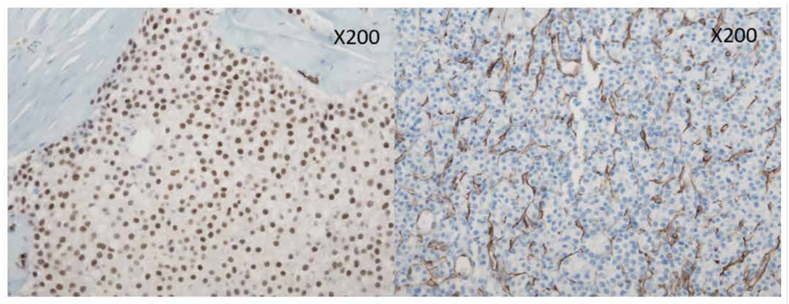
Hierarchical clustering of the entire samples were performed using 1,110 carcinoma-specific DEGs, and all carcinomas were completely clustered without misclassification. Further, we performed 2-axis DEG analysis with various cut-off combinations, and hierarchical clustering was performed with corresponding carcinoma-specific DEGs. As a result, only 597 carcinoma-specific DEGs found at the most severe cut-off (padj < 0.01 & log2 fold change > 2) were sufficient to completely classify carcinomas.

Top-ranked carcinoma-specific DEGs

Gene	Log_2FC (carcinoma)*	P_{adj} (carcinoma)*
MYH8	10.7	4.5×10^{-4}
WT1	10.2	6.6×10^{-5}
AHSP	10.1	1.8×10^{-3}
SLC17A8	9.2	6.1×10^{-4}
CHRM2	7.4	6.8×10^{-3}
GSG1L	7.3	9.2×10^{-3}
WDR64	7.3	6.0×10^{-7}
C1QL2	7.1	9.9×10^{-3}
HPD	6.7	2.0×10^{-4}
NMU	5.8	5.8×10^{-3}
ADTRP	-5.4	7.3×10^{-11}
PEBP4	-5.7	1.5×10^{-6}
CLEC2L	-6.1	1.3×10^{-9}
CFAP47	-6.2	2.6×10^{-10}
FLG	-6.8	3.2×10^{-3}
MYO3B	-7.2	2.7×10^{-21}
PIK3C2G	-7.3	5.5×10^{-15}
ALDH3B2	-7.8	3.9×10^{-11}
IL4	-12.3	2.4×10^{-4}
1TBXT	-14.8	8.7×10^{-4}

MYH8, WT1, and ASHP were observed as the most significantly up-regulated genes specifically for carcinoma, and down-regulation of MYO3B, PIK3C2G, and ALDH3B2 was also noticeable.

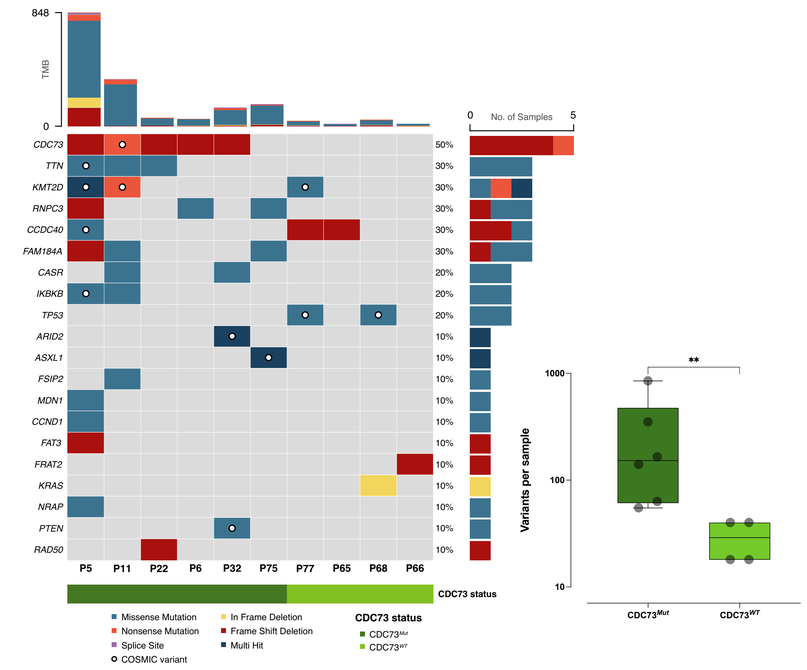


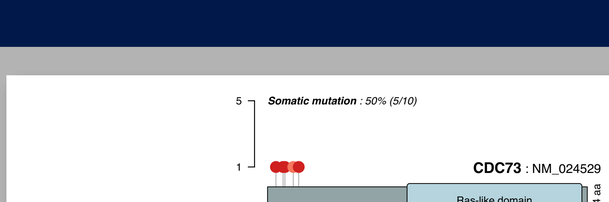


Upregulation on WT1 has been reported in several cancers, and its reproducibility is relatively high, making it easy to use as a simple molecular marker for diagnosis. Therefore, we further focused on WT1. The expression of WT1 was subdivided according to CDC73 mutation status, significance was found only in the CDC73Mut group. IHC staining result also showed WT1 positive only in CDC73Mut carcinoma samples (4 out of 7 CDC73Mut carcinomas), and none of CDC73WT carcinomas, adenomas, and normal tissues were stained.

Mutational landscape of parathyroid carcinoma

Among the somatic variants obtained from WES of 10 carcinoma DNA samples, only 9 mutated genes were observed in two or more samples, and the most frequent mutated gene was CDC73. (5 out of 10 carcinomas)

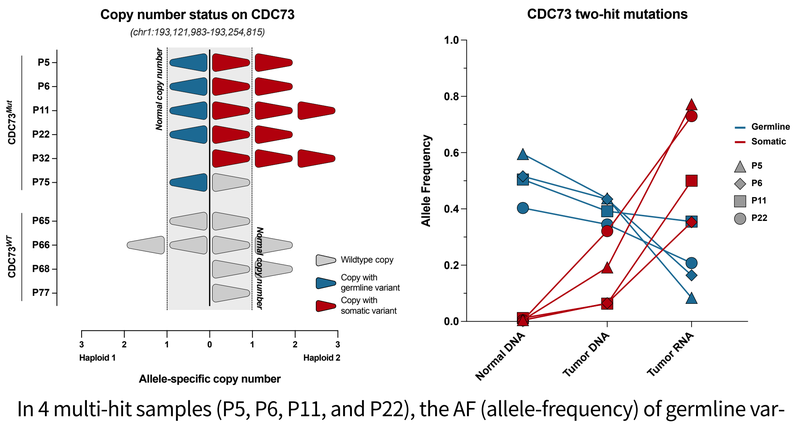




The CDC73 somatic mutations in these 5 samples were all truncating mutations such as nonsense or frameshift, and 4 of them (P5, P6, P11, and P22) were two-hit mutation that already had germline mutation at different position in CDC73. A germline mutation of CDC73 was also found in 5 samples, all of which were truncating mutations, and all found upstream of the gene. There were no highly repetitively mutated genes other than CDC73, but the mutations on KMT2D and CCDC40 were all damaging variant or COSMIC variant, suggesting that these genes may had some effect on the tumorigenesis. In the group of CDC73WT, somatic mutations on general tumor suppressor such as TP53 and KRAS, or WNT signaling pathway genes such as FRAT2 were found. The number of somatic variants found in the coding region was significantly higher in the CDC73Mut group, suggesting that the disruption of DNA repair mechanism is frequent.

Allele-specific expression of CDC73 in parathyroid carcinoma

For 10 carcinoma samples in this study, paired sequencing data sets of normal DNA, tumor DNA, and tumor RNA were collected, allowing us to examine copy number status and ASE (allele-specific expression) all at once. Therefore, we focused on the CNV and ASE of the CDC73, and observed a copy number gain on the CDC73 region in 6 out of 10 carcinoma samples, 5 of which had somatic mutation in CDC73.



In 4 multi-hit samples (P5, P6, P11, and P22), the AF (allele-frequency) of germline variant decreased as much as the AF of somatic variant, so it can be inferred that two mutations exist in different copies of homologous chromosomes, and the copy number gain occurred in the copy with somatic variant. This allelic imbalance due to the increased copy number of somatic mutant copy was even more severe in tumor RNA. In all 4 individuals, the proportion of transcripts with germline mutation was significantly reduced, whereas those with somatic mutation showed a significantly increased proportion. The LOH (loss of heterozygosity) of CDC73 was observed in 3 samples, (P32, P68, and P75) and in the case P75, a critical frameshift mutation was also found in the remaining copy of CDC73, so it can be expected that this sample also had CDC73-null effect in the same way as the two-hit mutation samples.

CONCLUSION

- Parathyroid carcinoma had distinctive transcriptional profiles compared to nomral parathyroid tissue and parathyroid adenoma.
- Carcinoma-specific DEGs including WT1 can function as distinctive diagnostic markers.
- There were no highly recurrent mutant genes other than CDC73.
- A two-hit mutation of CDC73 was found in many carcinoma patients, and the expression of late-hit copy was found to be asymmetrically high.

REFERENCES

1. Marcocci, Claudio et al. **"Parathyroid carcinoma."** Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research vol. 23,12 (2008): 1869-80.
2. Akirov, Amit et al. **"The Clinicopathological Spectrum of Parathyroid Carcinoma."** Frontiers in endocrinology vol. 10 731. 23 Oct. 2019
3. Cardoso, Luís et al. **"Molecular genetics of syndromic and non-syndromic forms of parathyroid carcinoma."** Human mutation vol. 38,12 (2017): 1621-1648.
4. Juhlin, C Christofer, and Lori A Erickson. **"Genomics and Epigenomics in Parathyroid Neoplasia: from Bench to Surgical Pathology Practice."** Endocrine pathology vol. 32,1 (2021): 17-34.
5. Hu, Ya et al. **"Circular RNA profile of parathyroid neoplasms: analysis of co-expression networks of circular RNAs and mRNAs."** RNA biology vol. 16,9 (2019): 1228-1236.

ACKNOWLEDGEMENT

This research was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (No. 2019R1A2C2008050).

YONSEI UNIVERSITY COLLEGE OF MEDICINE