Chinese Medical Journal®

Advances in the molecular genetics of parathyroid tumors

Liwei Zhao, Xianghui He

Department of General Surgery, Tianjin Medical University General Hospital, Tianjin 300052, China.

To the Editor: Parathyroid tumors are endocrine tumors that can lead to disorders of calcium and phosphorus metabolism and involve multiple organ systems. In the past, the genetics of parathyroid tumors have been well established, and the significant roles of genes such as *MEN1*, *CDC73*, and *CCND1* in the process of parathyroid tumorigenesis have been identified and confirmed [Table 1]. However, with further research and the widespread use of sequencing technology, "new" roles for "old" genes and new pathogenic mechanisms have been discovered.

The roles of the *MEN1* gene, one of the most commonly mutated genes in parathyroid tumors and encoding menin in the regulation of apoptosis, have been revisited in recent studies. In *MEN1*-associated pancreatic tumors, deletion of *MEN1* results in decreased caspase 8 and caspase 3 activity and downregulation of the levels of their cleaved forms of the proteins, as well as an accumulation of anti-apoptotic isoforms.[1] It may have the same effects in parathyroid tumors.

Parafibromin deficiency due to mutations of the *CDC73* gene is the most common cause of parathyroid carcinoma. Except for the mutation of *CDC73* gene, a recent study found that parafibromin may also be regulated by the ubiquitin-proteasome. They found that ubiquitin-specific proteases 37 (USP37) directly binds to parafibromin and prevents ubiquitin-induced degradation of parafibromin.[2] Thus, abnormalities of the ubiquitin-proteasome may also result in parafibromin dysfunction. In addition to stabilizing parafibromin, USP is also a negative regulator of the Wnt/β-catenin signaling pathway, which plays an important role in parathyroid tumorigenesis. USP7 can negatively regulate Wnt signaling by directly interacting with Axin through its tumor necrosis factor (TNF) receptor associated factor (TRAF) structural domain, promoting the deubiquitination and stabilization of Axin.[3]

The homonymous protein encoded by the enhancer of zeste homolog 2 (*EZH2*) is a histone methyltransferase that acts as an episodic silencing factor during tumorigenesis and progression. Recently, EZH2 was shown to play an important role in parathyroid development by regulating the expression of T-box transcription factor 1 (TBX1) and glial cells missing transcription factor 2 (GCM2).[4,5] EZH2 also interacted with micro RNA (miRNA)-101 and miRNA-124 to form a complex regulatory network, which inhibited apoptosis/cell cycle arrest and contributed to cellular dedifferentiation as well as the establishment of the tumor microenvironment, thus promoting local cancer cell invasion and immune escape.[6–8]

PRUNE2 is a newly identified tumor suppressor gene that inhibits the activity of Ras homeolog family member A (RhoA) through its BNIP-2 and Cdc42GAP homology (BCH) structural domain, thereby suppressing the transformation of cancer cells. It has been found that the *PRUNE2* gene can undergo germline or somatic mutations in parathyroid carcinoma, resulting in prune homolog 2 with BCH domain (PRUNE2) protein missing the BCH structural domain and losing its cancer-suppressive effect. Additionally, unlike *PRUNE2* mutations were found in the 18% of parathyroid carcinomas patients, mutations were rarely found in adenomas, which suggest that *PRUNE2* may be a potential marker to identify benign and malignant parathyroid tumors.[9]

TBX1 is a developmentally relevant gene, and deletion of *TBX1* during embryonic development can lead to hypoparathyroidism.[10] Significantly reduced *TBX1* expression was also found in parathyroid adenomas and carcinomas. It was also found that *TBX1* expression may be regulated by parafibromin. There was no significant difference in *TBX1* expression between parafibromin-positive cancer tissues and normal tissues, but the expression of *TBX1* was significantly reduced in parafibromin-deficient carcinoma tissues.[11] *EZH2* may also be a repressor

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000002935

Correspondence to: Xianghui He, Department of General Surgery, Tianjin Medical University General Hospital, Tianjin 300052, China
E-Mail: humphreyhe@163.com

Chinese Medical Journal 2024;137(3)
Received: 29-04-2023; **Online:** 11-01-2024 **Edited by:** Xiangxiang Pan

Table 1: Common genetic alterations in parathyroid tumors.

Gene	Encoded protein	Mutations	Encoded protein functions	Diseases	Related pathways
MEN1	Menin	Shift mutation, nonsense mutation, and missense mutation; 5′ UTR mutation	Regulates signaling pathways; Epigenetic regulation; Promotes apoptosis	Multiple endocrine neoplasia type 1; Sporadic parathyroid adenomas	Hedgehog pathway, TGF-β pathway, Wnt/β-catenin pathway, etc.
CCND1	Cyclin D1	Gene rearrangement (pericentric inversion); Gene amplification; Selective splicing	Promotes cell cycle progression; Promotes chromosomal instability; Regulates transcription factors; Interferes with DNA damage repair	Sporadic parathyroid adenomas; Sporadic parathyroid carcinomas	Wnt/β-catenin pathway
CDC73	Parafibromin	Frameshift mutation; 5′ UTR mutation	Regulation of transcript elongation and stability via interaction with the RNA polymerase II; Histone modification	HPT-JT syndrome; Sporadic parathyroid carcinomas	Wnt/β-catenin pathway
EZH2	EZH2	Gene mutation and amplification	Histone modification	Sporadic parathyroid adenomas; Sporadic parathyroid carcinomas	Wnt/β-catenin pathway
GCM2	GCM2	Gene activating mutation (CCID)	Related to parathyroid gland development; Activation of PTH transcription	FIHP; Sporadic parathyroid carcinomas	–
CDKN1B	P27	Gene mutations; Epigenetic silencing	Negatively regulates cell cycle and cell growth by inhibiting cyclin E/CDK2 complex	Multiple endocrine neoplasia type 4; Sporadic parathyroid adenomas	–

CCID: C-terminal conserved inhibitory domain; CDK2: Cyclin-dependent kinase 2; EZH2: Enhancer of zeste homolog 2; FIHP: Familial isolated hyperparathyroidism; GCM2: glial cells missing transcription factor 2; HPT-JT: Hyperparathyroidism-jaw tumor; MEN: Multiple endocrine neoplasia; TGF-β: Transforming growth factor beta; UTR: Untranslated region. –: Not avaiable.

of *TBX1* expression.[12] However, some experiments have reached the opposite conclusion–loss of *EZH2* or inhibition of its methyltransferase activity is associated with reduced *TBX1* expression.[4] Whether *TBX1* is a driver of parathyroid tumorigenesis or merely a target of other upstream genes (e.g., *CDC73*), the regulatory relationship among *EZH2*, *CDC73*, and *TBX1*, and the role that *TBX1* mutation plays in tumorigenesis still require extensive studies to explain.

A new differential gene *POMC* was identified by analyzing the transcriptome and DNA methylation profile of parathyroid adenoma. Pro-opiomelanocortin (POMC) is a precursor of several pituitary hormones (e.g., adrenocorticotropic hormone [ACTH]) and has an important regulatory role in the hypothalamic–pituitary–adrenal (HPA) axis. The structural similarity between parathyroid hormone (PTH) and ACTH leads to dysfunction of the HPA axis in PHPT patients. This link that exists between PHPT and the HPA axis suggests that POMC may influence primary hyperfunction of parathyroid (PHPT) via the HPA axis. Hypermethylation of CpG islands and low expression of the *POMC* gene were found in parathyroid adenoma tissues compared with normal parathyroid tissues, suggesting that hypermethylation of the *POMC* gene may be associated with the development of parathyroid adenomas.[13] This finding provides a new approach for investigating the pathogenesis of parathyroid adenomas by examining other endocrine axes.

High-frequency alterations in *KMT2D* (variant frequency ≥10%) were identified during whole-exome sequencing of Chinese patients with parathyroid adenomas. Mutations in *KMT2D* were found in 20.5% of patients with parathyroid adenomas, indicating that *KMT2D* might be a potential driver gene in the pathogenesis of parathyroid adenoma.[14] The *KMT2D* gene encodes a histone methyltransferase and epigenetic regulator that plays a crucial role in regulating gene transcription. It is currently thought to play a role as a repressor in tumorigenesis.

In recent years, with the widespread application of whole-exome sequencing technology, an increasing number of new gene mutations have been reported. For example, *ASXL3*, a recurrently mutated gene found in Chinese patients with parathyroid adenomas, which encodes a zinc finger structural domain-containing protein involved in the regulation of gene transcription. In addition, mutations of *ZFX, FAT1, FAT3, ADCK1, AKAP9* and other genes have also been detected. However, the frequency of mutations in these genes is relatively low, and it is not yet possible to determine whether there are meaningful stable mutations in parathyroid tumors and whether these mutations contribute to the development of parathyroid tumors.

Unlike genetic mutations, the process of epigenetic regulation is reversible. This feature provides more possibilities for the development of antitumor therapies targeting epigenetic regulation. Genes such as *MEN1*, *EZH2*, *CDC73*,

etc. mentioned above are also largely dependent on epigenetic regulation.

A new target for epigenetic regulation, the paired box 1 (*PAX1*) gene, was identified in recent studies. It was found that messenger RNA (mRNA) levels and protein expression of *PAX1* were significantly reduced in parathyroid adenomas tissue compared to normal parathyroid tissue. *PAX1* silencing was found to be associated with high methylation of the promoter region as well as histone deacetylation.[15] *PAX1* is associated with parathyroid development and can influence the regulation of *GCM2* gene expression, and its epigenetic silencing may be a potential cause of parathyroid tumorigenesis.

MicroRNAs are important components of epigenetic regulation and play an integral role in parathyroid tumorigenesis. For example, miR-24-1 inhibits the translation of MEN1 mRNA, leading to the deletion of Menin. Similarly, overexpression of hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) due to downregulation of miR-296 was found in parathyroid carcinoma, which led to abnormal activation of the Wnt/β-catenin signaling pathway, which promotes cell proliferation via activation of cyclin D1. In addition, aberrant miRNAs affecting the normal expression of genes, such as cyclin D1 (miR-17-5p), calcium-sensing receptor (miR-31-5p, miR-135b-5p), cyclin-dependent kinase inhibitors (miR-186-5p), and β-catenin (miR-330-3p), has been found in parathyroid tumors.[16]

With the advancement of detection technology, studies analyzing the miRNA profiles of different parathyroid tumors have also identified miRNA 199b-5p, which can be used to differentiate between hereditary or sporadic adenomas,[17] and potential markers of benign and malignancy such as miR-139, miR-222, miR-30b, miR-517c, and miR-126, among others.[18]

In summary, due to the rapid advances in sequencing and other technologies, more and more genetic alterations are being revealed in the process of parathyroid tumorigenesis, but the study of the mechanism of action of these "new" genes is currently insufficient and more in-depth studies are needed in the future.

Conflicts of interest

None.

References

1. Wagener N, Buchholz M, Bertolino P, Zhang CX, Di Fazio P. Exploring the MEN1 dependent modulation of caspase 8 and caspase 3 in human pancreatic and murine embryo fibroblast cells. Apoptosis 2022;27:70–79. doi: 10.1007/s10495-021-01700-1.
2. Kim SY, Lee JY, Cho YJ, Jo KH, Kim ES, Han JH, *et al*. USP37 deubiquitinates CDC73 in HPT-JT syndrome. Int J Mol Sci 2022;23:6364. doi: 10.3390/ijms23126364.
3. Ji L, Lu B, Zamponi R, Charlat O, Aversa R, Yang Z, *et al*. USP7 inhibits Wnt/β-catenin signaling through promoting stabilization of Axin. Nat Commun 2019;10:4184. doi: 10.1038/s41467-019-12143-3.
4. Caprio C, Lania G, Bilio M, Ferrentino R, Chen L, Baldini A. EZH2 is required for parathyroid and thymic development through differentiation of the third pharyngeal pouch endoderm. Dis Model Mech 2021;14:dmm046789. doi: 10.1242/dmm.046789.
5. Collinson A, Collier AJ, Morgan NP, Sienerth AR, Chandra T, Andrews S, *et al*. Deletion of the polycomb-group protein EZH2 leads to compromised self-renewal and differentiation defects in human embryonic stem cells. Cell Rep 2016;17:2700–2714. doi: 10.1016/j.celrep.2016.11.032.
6. Jin Q, He W, Chen L, Yang Y, Shi K, You Z. MicroRNA-101-3p inhibits proliferation in retinoblastoma cells by targeting EZH2 and HDAC9. Exp Ther Med 2018;16:1663–1670. doi: 10.3892/etm.2018.6405.
7. Jiang M, Xu B, Li X, Shang Y, Chu Y, Wang W, *et al*. O-GlcNAcylation promotes colorectal cancer metastasis via the miR-101-O-GlcNAc/EZH2 regulatory feedback circuit. Oncogene 2019;38:301–316. doi: 10.1038/s41388-018-0435-5.
8. Chen L, Jia J, Zang Y, Li J, Wan B. MicroRNA-101 regulates autophagy, proliferation and apoptosis via targeting EZH2 in laryngeal squamous cell carcinoma. Neoplasma 2019;66:507–515. doi: 10.4149/neo_2018_180811N611.
9. Yu W, McPherson JR, Stevenson M, van Eijk R, Heng HL, Newey P, *et al*. Whole-exome sequencing studies of parathyroid carcinomas reveal novel PRUNE2 mutations, distinctive mutational spectra related to APOBEC-catalyzed DNA mutagenesis and mutational enrichment in kinases associated with cell migration and invasion. J Clin Endocrinol Metab 2015;100:E360–E364. doi: 10.1210/jc.2014-3238.
10. Alghamdi M, Al Khalifah R, Al Homyani DK, Alkhamis WH, Arold ST, Ekhzaimy A, *et al*. A novel TBX1 variant causing hypoparathyroidism and deafness. J Endocr Soc 2019;4:bvz028. doi: 10.1210/jendso/bvz028.
11. Verdelli C, Avagliano L, Guarnieri V, Cetani F, Ferrero S, Vicentini L, *et al*. Expression, function, and regulation of the embryonic transcription factor TBX1 in parathyroid tumors. Lab Invest 2017;97:1488–1499. doi: 10.1038/labinvest.2017.88.
12. Singarapu N, Ma K, Reeh KAG, Shen J, Lancaster JN, Yi S, *et al*. Polycomb Repressive Complex 2 is essential for development and maintenance of a functional TEC compartment. Sci Rep 2018;8:14335. doi: 10.1038/s41598-018-32729-z.
13. Zhou WX, Wang S, Wu TC, Cheng LC, Du Y, Wu W, *et al*. Gene expression and methylation profiles show the involvement of POMC in primary hyperparathyroidsm. J Transl Med 2022;20:368. doi: 10.1186/s12967-022-03568-4.
14. Tao X, Xu T, Lin X, Xu S, Fan Y, Guo B, *et al*. Genomic profiling reveals the variant landscape of sporadic parathyroid adenomas in Chinese population. J Clin Endocrinol Metab 2023;108:1768–1775. doi: 10.1210/clinem/dgad002.
15. Singh P, Bhadada SK, Arya AK, Saikia UN, Sachdeva N, Dahiya D, *et al*. Aberrant epigenetic alteration of PAX1 expression contributes to parathyroid tumorigenesis. J Clin Endocrinol Metab 2022;107:e783–e792. doi: 10.1210/clinem/dgab626.
16. Yavropoulou MP, Pazaitou-Panayiotou K, Yovos JG, Poulios C, Anastasilakis AD, Vlachodimitropoulos D, *et al*. Circulating and tissue expression profile of MicroRNAs in primary hyperparathyroidism caused by sporadic parathyroid adenomas. JBMR Plus 2020;5:e10431. doi: 10.1002/jbm4.10431.
17. Hwang S, Jeong JJ, Kim SH, Chung YJ, Song SY, Lee YJ, *et al*. Differential expression of miRNA199b-5p as a novel biomarker for sporadic and hereditary parathyroid tumors. Sci Rep 2018;8:12016. doi: 10.1038/s41598-018-30484-9.
18. Hu Y, Zhang X, Cui M, Su Z, Wang M, Liao Q, *et al*. Verification of candidate microRNA markers for parathyroid carcinoma. Endocrine 2018;60:246–254. doi: 10.1007/s12020-018-1551-2.

How to cite this article: Zhao LW, He XH. Advances in the molecular genetics of parathyroid tumors. Chin Med J 2024;137:376–378. doi: 10.1097/CM9.0000000000002935