Research article

Recent Advances in Genetic Mutations in Papillary Thyroid Carcinoma

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ABSTRACT

Papillary thyroid carcinoma (PCT), follicular origin are divided into RAS-like malignancies and BRAF-like malignancies according to their molecular characteristics and aggressiveness. With the continuous development of the molecular level, genetic mutations are significant in the diagnosis, treatment and prognosis of papillary thyroid carcinoma. This paper discusses the latest progress of papillary thyroid cancer gene mutation and its clinical application, in order to provide more efficient diagnosis and treatment means for patients.

Key words: Papillary thyroid carcinoma, gene mutation, gene fusion, targeted drugs
0 Introduction

Thyroid cancer (thyroid carcinoma, TC) is the most common malignancy in the endocrine system and head and neck tumors, Common types include: papillary carcinoma (Papillary Carcinoma of Thyroid, PCT), follicular carcinoma (follicular thyroid cancer, FTC), medullary carcinoma (medullary thyroid carcinoma, MTC) and undifferentiated carcinoma (anaplastic thyroid cancer, ATC), Of which PCT was the most common primary thyroid tumor, Accounting for 74-80% of all thyroid cancers[1]. It can be seen in all ages, and the ratio of male to female incidence is about 1:3[2]. Classical PTC often has an indolent clinical course[3], Morbidity and, with lower mortality[4], However, there are still patients with extradandular invasion, lymph nodes, lung, brain metastasis, etc., leading to poor prognosis due to recurrence or metastasis[5]. Genetic and other factors are one of the common causes of PTC[6], The 5th edition WHO classification of thyroid tumors combines tumor morphological features, mutation, and transcriptomic features to propose RAS-like and BARF-like tumors[7], Gene and biological characteristics provide precise guidance for the targeted treatment of papillary thyroid carcinoma, and this paper summarizes the latest development of papillary thyroid carcinoma gene mutation and its clinical significance.
Figure 1 A common type of Thyroid cancer

1 RAS Gene Mutations

The Ras protein in the mitogen-activated protein kinase (MAPK) signaling pathway is one of the most frequently mutated oncogenes in cancer[8]. Approximately 19% of humans carry mutations, with an estimated 3.4 million new cases occurring annually worldwide[9]. Ras, a GTP-GDP binary switch protein, transduce signals from different receptors to regulate various signaling networks. The "three brothers" of the Ras gene family are Harvey (H-Ras), Kirsten (K-Ras, with two splice variants K-Ras4A and K-Ras4B), and neuroblastoma (N-Ras)[10]. Ras proteins receive the upstream signal receptor tyrosine kinase (tyrosine kinase, TK) by binding to the guanine nucleotides (Guanosine5′-monophosphate, GMP) and cycle between active guanosine diphosphate (GTP) and inactive, guanosine triphosphate (GDP) conformations to activate the downstream MAPK pathway[8], Lead to abnormal cell proliferation, resulting in the occurrence of cancer.

With the advent of next-generation sequencing (NGS) and other sequencing technologies, there are more and more studies based on PTC
mutations. A study based on PTC population sequencing data in Shandong, China showed that the RAS mutation rate in PTC patients was 10.2%[11], This is significantly lower than the global average RAS, with a mutation rate of 19%[9], The main reason for this is the different population base. Compared with PTC, RAS mutations are more frequently in FTC, including PI3K-AKT, RASSF1-MST 1-FOXO3 signaling, and PAX 8 / PPARG fusion[12]. And Mengdi Jin et al[13] Genotyping of the six SNP of RAS (KRAS-rs12427141, KRAS-rs712, KRAS-rs7315339, HRAS-rs14804 and NRAS-rs2273267) using matrix-assisted laser, The interaction between KRAS-rs12427141 and HRAS-rs12628 was found to increase the risk of PTC (U= -2.119, p<0.05), The interaction between KRAS-rs2273267 and HRAS-rs7315339 reduced the risk of PTC (U=2.195, p<0.05, This suggests PTC-related interactions between RAS family gene polymorphisms in the Chinese Han population, Provide clues to the pathogenesis and potential therapeutic targets of PTC.

The best treatment is direct inhibition of mutated RAS by allele-specific inhibitors. Therapies targeting the RAS activation pathways or RAS effector pathways can be combined with RAS inhibitors, immune checkpoint inhibitors, or T cell-targeting approaches to treat RAS-mutant tumors[14]. The protein of Christine rat sarcoma (KRAS), encoded by the KRAS gene, is a signal transduction that plays a crucial role in regulating
cell proliferation[15], The US Food and Drug Administration (FAD) approved cabotinib as a targeted drug of potential benefit. Novel pan-KRAS inhibitors targeting the KRAS-SOS1 interaction by computational modeling were developed by Patel et al[15], The establishment of this model provides a new means for the treatment of clinical PTC.

2 BRAF V600E Gene Mutation

BRAF (rat fibrosarcoma (Raf) mouse sarcoma virus oncogene B) is a proto-oncogene located on chromosome 7 with a prevalence of 45% to 90% in PTC, and is the most common mutation in PTC[16], V600E is a driver mutation in the BRAF proto-oncogene, named because valine (V) was replaced by glutamate (E) at amino acid 600[17]. Mutant BRAF results in hyperactivation of the MAPK pathway and uncontrolled cell growth[18].

![Figure 2. The details of one of type Mutation in BRAF](image-url)
BRAF V600E Gene mutations are associated with an aggressive phenotype, enabling, faster tumor growth and higher mortality[17]. Metastasis or recurrence can lead to a poor prognosis in PTC, but studies have shown that[19], Neck lymph node metastasis (LNM) in PTC patients was associated with clinicopathological features such as maximum tumor diameter, number of primary lesions, and vascular invasion, but not with BRAF V600E mutation. Similarly, the BRAF V600E mutation was associated with advanced age and the aggressiveness of PTC, independent of LNM, as found by Deng C et al. And PTC with BRAF V600E mutation may have higher invasive siveness[20]. This suggests that BRAF V600E predicts cervical lymph node metastasis or acts as a risk stratification factor for PTC, which remains to be extensively studied. Fine-needle aspiration cytology (FNAC) is a non-invasive method that is considered the most cost-effective and accurate diagnostic method for the diagnosis of PTC[21]. A study of BRAF V600E mutation frequency in patients with PTC in China[22]Found that in thyroid nodules with indeterminate cytology, BRAF mutation detected PTC with sensitivity of 87.69% and specificity as high as 100.00%, which has high diagnostic value for thyroid nodules with indeterminate FNAC. Furthermore, an Indonesian study showed that[23], 41.3% carried KRAS, mutation, or EGFR mutations in any of the BRAF V600E-negative samples. This suggests that for BRAF V600E
negative samples, testing of RAS or EGFR mutations may be required to further consider treatment.

The current selective BRAF kinase inhibitor Dabrafenib, in combination with the MEK 1/2 inhibitor Trametinib, has been approved by the FDA for the treatment of tumors containing specific BRAF V600E, mutant solid tumors[24]. Vemurafenib is also a specific inhibitor of BRAFV600E kinase, but it is usually limited by short-term responses and acquired resistance by heterogeneous feedback mechanisms. Copper-dependent disulfiram (DSF / Cu) kills and sensitize BRAF mutant thyroid cancer cells with feedback activation of MAPK / ERK and PI3K / AKT pathways[25]. In addition, the investigators found that the[26], BRAFV600E and cytochrome p4502s1 antibodies (CYP2S1) are mutually reinforcing and are synthetic lethal partners. LiY et al., through targeted delivery of CYP2S1-specific siRNA, demonstrated that CYP2S1 is a potential therapeutic target in BRAFV600E-driven xenograft and transgenic mouse models. All of these provide new therapeutic strategies for BRAFV600E-mutated PTC.

4 RET gene fusion

RET, which is a proto-oncogene located on the long arm of chromosome 10 (10q11.21), is a member of the cadherin superfamily and encodes the RET protein that is a TK[27], Can participate in the PI3K-AKT-mTOR
pathway as well as the RAS-RAF-MEK-ERK pathway to induce cellular hyperplasia. RET often uses the 3’ RET sequence encoding TK to the 5’ sequence from other genes to reconstitute a new gene with self-phosphorylation and continuous activation. RET fusions are reported to be present in 1 – 2% of lung adenocarcinomas and in 10 – 20% of PTC[28] Among them, CCDC 6-RET fusion and NCOA4-RET fusion formed by coiled-coil domain family 6 (CCDC 6), nuclear receptor co-activator 4 antibody (NCOA4) and RET gene are the most common RET gene fusions in PTC patients[29]. Recently, Khonrak T et al found that the presence of CCDC 6-RET fusion was significantly associated with absence of classical subtype and vascular / lymphatic vessel infiltration (p <0.05) and NCOA4-RET was associated with an aggressive phenotype of PTC[29]. In addition, the Pekova B[30] And others found that in pediatric PTC, RET fusion was associated with more frequent lymph node and distant metastases as well as sand particles, all suggesting that RET fusion is closely associated with the clinicopathological phenotype and could be used as a predictive marker in PTC patients. In addition, Krishnan A et al. identified a novel RET fusion-TK fusion in tumor material from patients who do not carry any known RAS or BRAF mutations, and showed that its expression upregulates E3 ubiquitin ligase (HUWE 1) to mediate tumorigenesis. Thyroid cancer patients with RET fusion mutations belong to BARF like in the fifth version of the WHO
classification[7], And are more likely to relapse than in patients without BRAF and RET fusion mutations. Selpercatinib is a specific kinase inhibitor approved by the FDA with pratinib for the treatment of thyroid cancer patients with positive RET fusion[32].

5 TERT Promoter Mutations

Promoter mutations in the telomerase reverse transcriptase (TERT) gene change the cytosine (C) at both sites to thymine (T), namely C228T and C250T, which plays an important role in PTC recurrence and disease-specific mortality[33]. Mutations of the TERT promoter lead to an upregulation of TERT mRNA expression, which in turn enhances telomerase activity, leading to unlimited proliferation of tumor cells. As found by Na H Y et al[34], In PTC, aggressive clinicopathological features, higher stage and BRAF V600E mutations were all found to be associated with TERT promoter mutations, and distant metastases and disease recurrence were more common in PTC with TERT promoter mutations. Same as the results for the investigator[35]Using a logistic regression analysis of the Cox proportional hazards model, we indicated that TERT promoter mutation was an independent predictor of distant metastasis of micropapillary thyroid carcinoma (PTMC) in the Middle East. In addition, the Choi J H[36] et al reported a case of a patient with PTC skin metastasis whose ThyroSeq molecular test showed both BRAFV600E mutation and TERT promoter C228T mutation, indicating
that patients coexisting with these two genes have a poor prognosis and long-term monitoring of disease recurrence is particularly important. However, due to the unknown clinical significance of this gene mutation, treatment has become a challenge, the development of telomerase inhibitors is extremely challenging, and so far there is no clinically approved strategy to use this cancer target[37]. Currently, chemical inhibition of TERT promoter mutations, immunotherapy is a therapeutic approach for TERT promoter mutations[38].

6 Other Genetic Mutations

In addition to the above gene mutations, PTC also has other kinds of gene mutations, such as PAX8 / PPARγ chromosome rearrangement, TSHR gene mutation, PIK3CA gene mutation, T P 53 gene mutation, etc. Mutations in the subunits of the SWI / SNF chromatin remodeling complex are common in cancers of different lineages, including late PTC, as the investigators found[39], SWI / SNF complex mutations, which are essential for maintaining the differentiation function of TC, and their absence leads to radioiodine refractory and resistance to MAK-based redifferentiation therapies with MAPK inhibitors. Analysis of gene mutations in papillary thyroid carcinoma in China are shown[40], High frequencies of mutations were detected in the RBM 10 gene (44%) and TERT (43%), and some hotspot mutations were found, indicating that deep sequencing of the Ultra-small gene panel can identify some low-
frequency mutated genes to provide targeted therapy for patients. Ananaplastic lymphoma kinase (ALK) fusions are rare in PTC but may lead to more aggressive behavior. Lee H[41] reported an ALK fusion — CCDC149-ALK not previously described in PTC and, in addition, Stosic A[42] et al tested candidate genes on PPTC specimens and revealed two previously undescribed fusions, STRN-RET and TG-PBF. Deep mutation sequencing of newly discovered genetic mutations should be performed to identify genetic alterations that can be targeted to provide help in the treatment of patients.

7 Conclusions

In conclusion, the continuous development of molecular biology technology has promoted the research and application prospects of PTC gene mutations. The mechanism and targeted medication of RAS, BRAF and RET gene mutations have been thoroughly studied, but there is still considerable room for progress in the clinical significance and targeted therapy of TERT promoter mutations. Deep sequencing can detect low-frequency mutations in PTC patients in order to find new tumor markers to predict tumor occurrence and provide help for the individualization of therapeutic strategies. With the continuous development of precision medicine, PTC gene mutation analysis will be more routine, which is expected to provide patients with more accurate and individualized diagnosis and treatment plans, so as to improve the quality of life and
prognosis of patients. At present, there has been a controversy over overtreatment of TC. How to find a balance between radical and conservative treatment plan will become the focus of scholars.

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