BRAF gene mutation and papillary thyroid carcinoma

Junru Zhang *

School of life science and technology, China Pharmaceutical University, Nanjing, China

* Corresponding Author Email: 2020201997@stu.cpu.edu.cn

Abstract. Nowadays, Thyroid carcinoma (TC) is the most common malignant tumor of endocrine system, accounting for about 1% of the malignant tumors in the whole body, ranking first in the head and neck malignant tumors. Papillary thyroid carcinoma (PTC) is a malignant tumor originated from thyroid follicular epithelial cells, which is the most common pathological type in TC. V-RAF murine sarcoma viral oncogene homolog B1 (BRAF) is a molecular biological marker for the diagnosis and prognosis of PTC and a target gene for the treatment of PTC. Its gene mutation is a hot point in the TC research field. The most common gene mutation is BRAF V600E mutation and it is a molecular marker in PTC. With the deep-going of its research, contradictions and controversies about the relationship between BRAF V600E and PTC gradually emerge. Many molecular targeted drugs targeting BRAF gene are also being continuously researched and some achievements have been made. This review mainly expounds the relationship between BRAF V600E and clinicopathological features, analyzes and expounds the controversial parts currently as well as show the research status of molecular targeted drugs.

Keywords: PTC, BRAF V600E, clinicopathological characteristic.

1. Introduction

TC is the most common malignant tumor of endocrine system, accounting for about 5% of all thyroid nodules and 1% of systemic malignant tumors. In 1960, Lindsay first described the unique characteristics of PTC, which made follow-up researchers pay attention to and distinguish this cancer type from other types for research.[1] Nowadays, according to WHO pathological classification, TC mainly includes the following four categories: follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC), PTC and anaplastic thyroid cancer (ATC). FTC and PTC are collectively referred to as differentiated thyroid carcinoma (DTC). The most common pathological type is PTC, accounting for more than 90% of TC. [2] The occurrence and development of TC includes a series of high-risk molecular genetic events, such as Ras gene mutation in FTC, RET / PTC gene rearrangement related to PTC and some thyroid adenomas, and p53 gene mutation in ATC. [3] In 2014, The Cancer Genome Atlas research network confirmed PTC features a BRAFV600E-like profile [4], indicating that BRAF V600E has a high incidence in PTC. [3]. Before 2000, PTC was considered to be lack of proper medical history and manifestations. [5]. In 2003, Cohen et al. [6] first found BRAF V600E gene mutation in TC, and subsequent studies have found that BRAF V600E is the most common molecular genetic change in TC [7]. In recent years, increasingly attention has been paid to PTC related molecular level research. Many studies have confirmed that BRAF V600E mutation detection based on molecular pathological diagnosis technology is closely related to PTC and can be regarded as a potential marker of PTC [8]. And its research is the most in-depth in recent years. [9] This article introduces BRAF gene and its BRAF V600E mutation types, and summarizes the research status and progress of BRAF V600E in PTC, in order to further explore the relationship between BRAFV600E mutation and PTC.

2. BRAF gene and protein

2.1. MAPK signaling pathways

The MAPK signaling pathway is an important signal transduction system which mediates cell life activities such as cell oxidative stress, cell proliferation and cell apoptosis. It can transmit the relevant

biological signals outside the cell to the inside of the cell and amplify these signals step by step at the same time, in order to stimulate the effector molecules in the cell to complete various life activities of cells. [10] The activated signaling pathway is carried on in the way of a three-stage kinase cascade (Fig. 1). This three-stage kinase cascade is consisted of a MAPK kinase kinase (MAPKKK, MKKK), a MAPK kinase (MAPKK, MKK) and a MAPK. [11] The ERK kinase family pathway is the classical pathway of the MAPK signaling pathway, its MAPKKK-MAPKK-MAPK three-stage kinase cascade is Ras-Raf-MEK-ERK pathway. The extracellular related signals activate the ERK signal pathway through step-by-step activation. The phosphorylated ERK goes into the nucleus and activates many transcription factors, such as Elk-1, ATF, NF-κB, c-fos, Ap-1, c-Jun and so on. These transcription factors further regulate the expressions of various proteins and take part in many biological reactions such as cell morphology maintenance, cell proliferation, cell differentiation and so on. [10]

The Raf family is belonging to MAPKKK and is highly conserved serine/threonine kinase, which is activated by the interaction with the Ras protein. The members of the Raf family include A-Raf, B-Raf and Raf-1(c-Raf or c-Raf-1). [12]

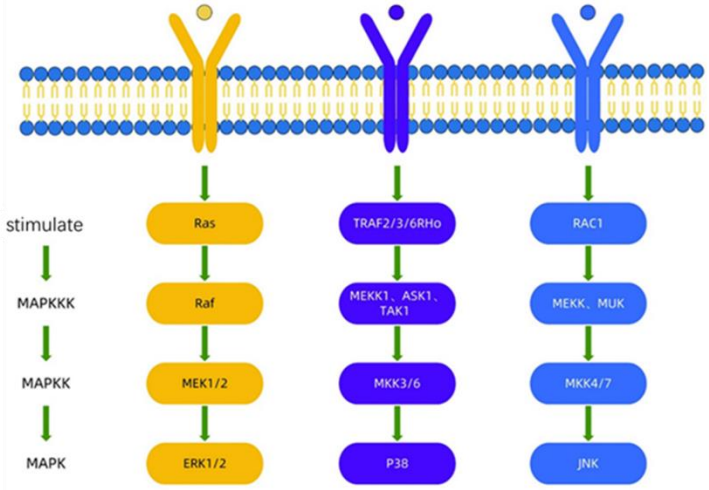


Figure 1. MAPK signaling pathways [10]

2.2. BRAF gene

BRAF gene was first discovered, cloned and identified in Ewing's sarcoma by Ikawa et al in 1988. It is named BRAF because it has quite high homology with A-raf and C-raf. BRAF is one kind of RAF gene family and is located on 7q34 of human chromosome. It contains 18 exons and is about 190kD in size, 94 kD among them can encode protein molecule—a 67~99 kD serine/threonine kinase, which has 783 amino acid residues. This serine/threonine kinase has a major part to play in the transmission of MAPK signaling pathway and is an important kinase in MAPK signaling transmission pathway RAS→RAF→MEK→ERK: the downstream protein kinase of RAS and RET (Fig. 2) [12,13]

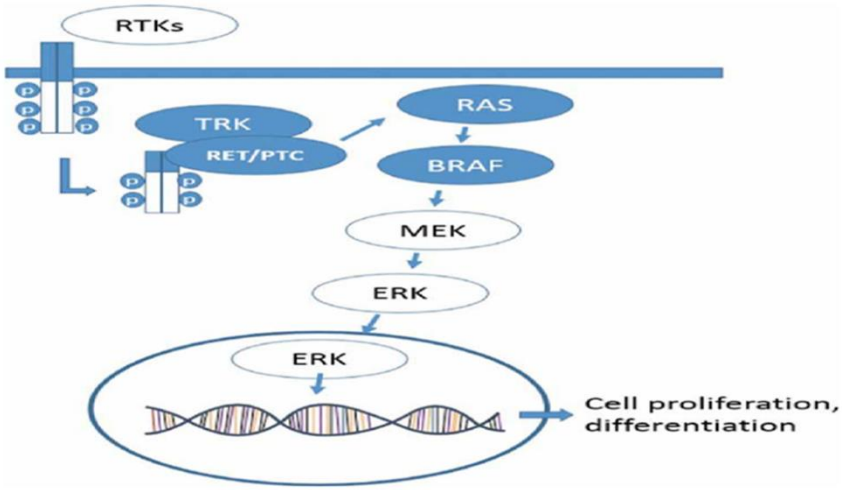


Figure 2. RAS/RAF/MEK/ERK signaling pathway[12]

2.3. BRAF protein structure

The 3D structure of BRAF protein is shown in Figure 3. BRAF protein contains three conserved regions: regularly regions and kinase domain CR3 from N-terminal and C-terminal. CR1 domain is rich in cysteine and is including RAS protein binding region and zinc finger motif. CR2 domain is rich in serine/threonine and is the phosphorylation regulatory region of the BRAF kinase. Its amino acid 361 - 365 region is a 14-3 -3 protein binding region, which stabilizes the inactive BRAF. The CR3 is necessary for the activation of the BRAF protein and it contains tyrosine and serine residues whose phosphorylation modification also change the activity of BRAF kinase. Among them, lysine at position 482 is the binding site of ATP, and amino acid 725-729 is another binding site with 14-3-3 protein. [11]

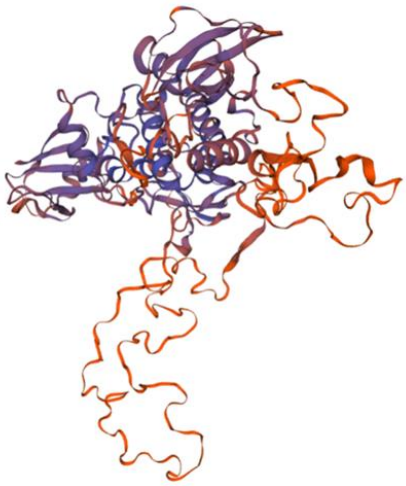


Figure 3. BRAF protein structure

2.4. BRAF protein activation

BRAF is activated mainly by the following two ways. First, when growth factors bind to their tyrosine kinase receptors on the cell surface, the two tyrosine kinase receptors dimerize and at the same time, their tyrosine are phosphorylated. This phosphorylation site can anchor the downstream adaptor protein which is the growth factor receptor binding protein. Then this protein connects to the exchange factor SOS, to let the information obtained by the receptor be transmitted into cells. SOS then activates the RAS protein downstream, make BRAF bind to RAS guanosine triphosphate complex which is converted from RAS guanosine diphosphate complex. At this time, the BRAF accumulates from the cytoplasm to the inner side of the cell membrane and is activated in many steps. Second, the growth factors can also activate the adenylate cyclase through the G protein coupled

receptor on the cell surface, making the cAMP inside the cell increase, and activates BRAF through G protein RAS or Rap1. However, the increase of cAMP inhibits the activity of the Raf-1. In the end, these two Raf proteins regulate the downstream ERK together. It is known that only few cells activate their BRAF through this pathway. The activated BRAF then phosphorylates and activates the downstream MEK and ERK in turn, and the ability of BRAF to activate the MEK is the strongest among the three Raf proteins. [11]

3. BRAF V600E gene mutation

3.1. 3.1 BRAF V600E gene mutation site

To this day, more than 40 types of BRAF mutations have been found. The most common one among these is BRAF V600E mutation, which is happening at 1799 mutation site in exon 15. This kind accounts for more than 90% of all BRAF mutations and leading to the valine, the one which is corresponding to codon 600 of the coding protein, substituted by glutamine. The mutations happening at other sites are relatively rare. [9,12] From the structure of the protein, the hydrogen bond interaction between the mutated glutamic acid and the main chain and side chain is increased because valine is hydrophobic and glutamic acid is hydrophilic. V600E mutation destroys the hydrophobic interaction between glutamic acid and the surrounding hydrophobic amino acid residues, affecting the protein structure. (Fig. 4, left: interaction between valine and protein main chain, right: interaction between valine and protein side chain. Fig. 5, left: interaction between glutamic acid and protein main chain, right: interaction between glutamic acid and protein side chain.) The BRAF mutations have inducing effects to the formations of many cancers, and the thyroid cancer is one of them. [13] The most common genetic event in the process of TC arising is BRAF V600E, whose incidence in PTC is the highest, up to 29%~83%. [9]

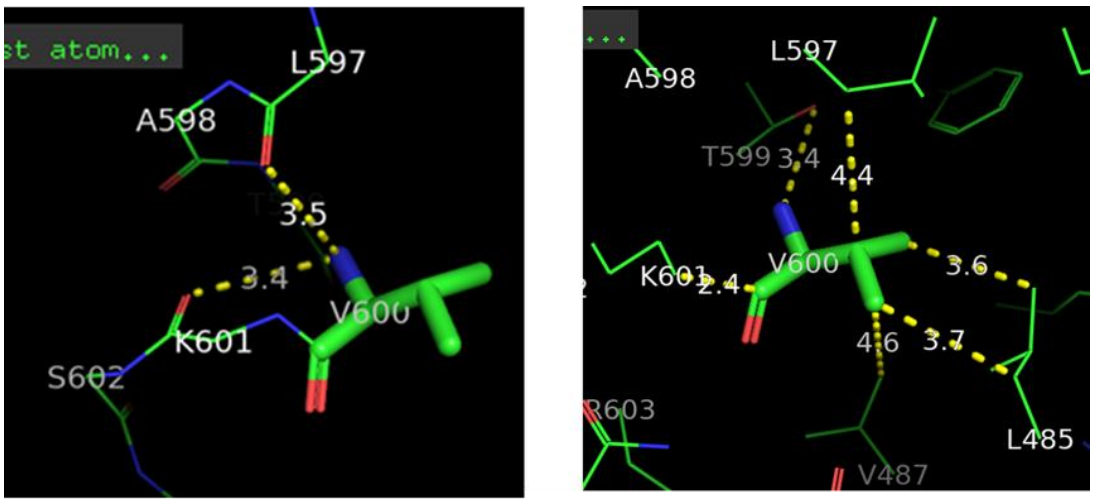


Figure 4. Left: interaction between valine at position 600 in BRAF protein and protein main chain; right: interaction between valine at position 600 in BRAF protein and protein side chain

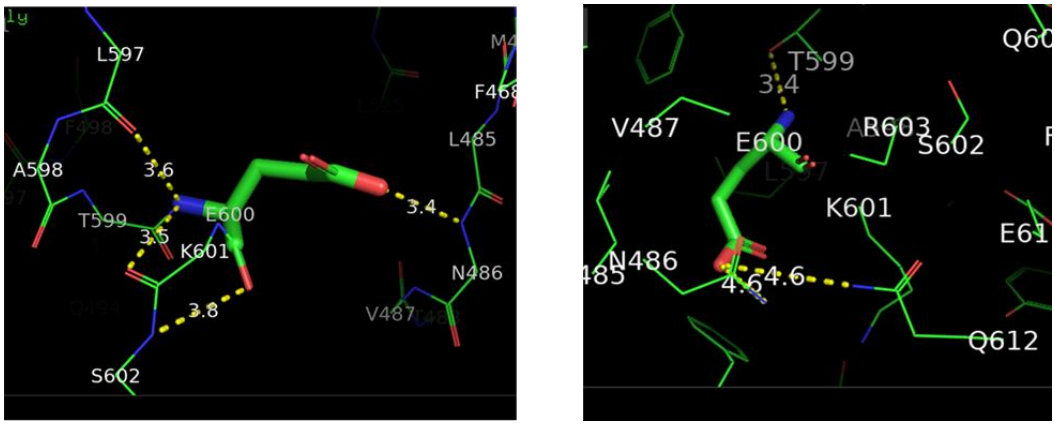


Figure 5. Left: interaction between glutamate at position 600 in BRAF V600E protein and protein main chain; right: interaction between glutamate at position 600 in BRAF V600E protein and protein side chain

3.2. Discussion on whether gender and age are the influencing factors of BRAF V600E mutation

Some ideas indicate that age and sex are related to the mutation of BRAF V600E gene, but at present, there is a great controversy about the relationship between BRAF V600E and the age and gender of PTC patients. After studying the clinical data of 115 PTC patients, Li Liping et al. [14] pointed out that the mutation rate of BRAF V600E gene detected in female PTC patients was significantly higher than that in male PTC patients, while the mutation rate had no significant correlation with age. However, Zeng Xunxiong et al. [15] obtained the opposite conclusion with Li Liping. They extracted the paraffin specimens DNA of 42 patients' paraffin PTC and finally, it was detected that there were 19 patients with BRAF V600E and the age of patients with this mutation was younger, but there was no significant difference between male and female patients. Yu Jing et al. [16] detected the mutation of BRAF V600E gene in 104 cases of PTC, and Lai Yeqian [17] screened the literature on patients with PTC who were tested for BRAF V600E before March 2014 from major well-known databases, in the end a total of 23 literatures with a score of 3~5 stars according to the Newcastle Ottawa scale (full score of 9 stars) and 2771 patients with PTC were selected. Then the reliable data in the literature were extracted by RevM an5.1 Conduct meta-analysis on the data. All of them suggested that the BRAF V600E had nothing to do with the patients' age and gender. Therefore, the correlation between BRAF V600E and age and gender needs to be further researched and analyzed.

3.3. Effect of BRAF V600E gene mutation

After the mutation of BRAF V600E, the RAS-RAF-MEK-ERK pathway will continue to be activated. The downstream controls on regulating the signals of cell differentiation, cell proliferation and cell apoptosis are released and the ability of cell mitosis is enhanced by this mutation. In the end, it makes cells proliferate abnormally and induces the occurrence of the tumor. [2,9]

The BRAF V600E mutation can also lead to the deacetylation of histone of sodium iodine transporter (NIS) gene promoter, resulting in the compaction of chromatin which blocks the combination between gene promoter and transcription factor, and makes the NIS gene silence. Then because of these, the expressions of sodium iodine transporter in cell membranes significantly decrease. Compared with the PTC tissue without mutation, the expression level of NIS in BRAF V600E mutation tissue is lower, reducing the iodine uptake ability of PTC cells which leads to poor therapeutic effects of radioactive iodine (RAI) and adds difficulties to tumor treatments. At the same time, there are studies which have found that the BRAF V600E can also promote the expression of transforming growth factor β and the increased expression further strengthens the iodine resistance and invasiveness of PTC. [2,9]

In addition, in the cells accompanied with BRAF V600E, the thyroid stimulating hormone (TSH) will strengthen the invasiveness of cells and instability of genome. At the same time, it is found that the BRAF V600E will aggravate the methylation of thyrotropin receptor (TSHR) gene promoter, causing the silence or notable drop of TSHR expression. Then the feedback of the TSH increases and the growths of tumor cells are promoted. [2,9]

4. Relationship between BRAF V600E and clinicopathological characteristic of PTC

BRAF V600E not only has a major part to play in the occurrence and development of tumors, but is closely related to the adverse clinicopathological characteristic of PTC as well, including tumor tissue size, clinical stage, local recurrence, distant metastasis and so on. However, this relationship is still controversial. [9] Now the following part of the article will discuss the relationship between BRAF V600E and these indexes.

4.1. BRAF V600E gene mutation and extra-thyroid infiltration

Extra-thyroid infiltration is one of the important poor prognostic factors of PTC. [9] Some studies have indicated that BRAF V600E is not associated with extra-thyroid infiltration, for example, the research results of Zang Xuxiong et al. [15] showed that BRAF V600E and extra-thyroid infiltration were not statistically significant. However, only 42 PTC patients were included in his study. The sample is too small, causing the results may be controversial. There are more studies support that the existence of BRAF V600E has a significant correlation with extra-thyroid infiltration. Wang Weina et al. [18] by the way of retrospectively analyzing the clinicopathological data of 110 PTC patients and 38 benign thyroid lesions patients. At the same time, they found that BRAF V600E was related to extra membranous invasion of thyroid gland. The research results of Li Linping et al. [14] indicated that the invasion rate of BRAF V600E PTC patients was significantly higher than that of PTC patients without BRAF V600E. Yu Jing et al. [16] announced that BRAF V600E was associated with extrathecal invasion. Zhang Wei et al. [19] used nested PCR to detect BRAF V600E in 55 PTC patients. The results showed that BRAF V600E was related to envelope infiltration in patients with PTC. Therefore, it can be considered that BRAF V600E is related to extra-thyroid infiltration.

4.2. BRAF V600E gene and lymph node metastasis

PTC is often associated with cervical lymph node metastasis, including central and lateral lymph node metastasis, of which central lymph node metastasis is more common. [9] Wu Wennian et al. [8] analyzed 387 BRAF V600E PTC patients and gained the results that BRAF V600E was statistically significant with cervical lymph node metastasis of PTC, which may represent that BRAF V600E is related to invasive biological behavior of PTC. It can provide some reference value for early diagnosis, selection of operational scheme and prognosis judgment of patients in clinic. Li Linping et al. [14] researched and showed that the rate of cervical lymph node metastasis in BRAF V600E PTC patients was significantly higher than that in patients without BRAF V600E. Lai Yeqian [17] found that the lymph node metastasis rate of BRAF V600E PTC patients was 54%, which was significantly higher than the 36% lymph node metastasis rate of non-mutated patients ($P>0.05$). The BRAF V600E has an influence on the lymph node metastasis rate of PTC patients. Zhang Wei et al. [19] found that BRAF V600E was associated with cervical lymph node metastasis in PTC patients. However, some researches showed that it is not found the correlation between BRAF V600E and lymph node metastasis. [18] Zhang Peng et al. [20] studied and showed that there was no significant difference in BRAF V600E mutation rate between those with and without cervical lymph node metastasis, suggesting that BRAF V600E may not be significantly related to PTC cervical lymph node metastasis. They thought the reason may be that BRAF V600E mainly occurred in the early growth of PTC tissue, while cervical lymph node metastasis occurred later. There is no direct correlation between them in the time sequence of tumor progression. However, the number of samples in these two studies is

small, including 110 PTC patients [18] and 34 PTC patients [20], so the reliability of their research conclusions needs to be confirmed by more in-depth research.

4.3. BRAF V600E gene mutation and TNM clinical stage

Whether BRAF V600E is related to TNM clinical stage is also a controversial issue. Lots of studies have reported that BRAF V600E is significantly correlated with TNM clinical stage. Wang Xuhong et al. [21] detected the mutation of BRAF V600E and TRET promoter in 728 PTC patients, and found that BRAF V600E was significantly correlated with TNM stage (P=0.009) and higher stage (P=0.011). Wang Weina et al. [18] indicated that BRAF V600E was related to high clinical stage (P<0.05). Li Linping et al. [14] pointed out that the TNM stage of BRAF V600E PTC patients was significantly higher than that of patients without BRAF V600E. However, there were also some researches showed that there was no association between the BRAF V600E and TNM clinical stage. [9,15,19]

4.4. BRAF V600E gene mutation and tumor diameter

A large number of studies have reported that BRAF V600E has no correlation with tumor diameter. Zang Xunxiong et al. [15] believed that BRAF V600E has no statistical significance with tumor size. Wang Weina et al. [18] and Zhang Wei et al. [19] both indicated that BRAF V600E had nothing to do with tumor diameter. But there are some researches shown a correlation between the two. Wang Xuhong et al. [21] pointed out that the BRAF V600E was significantly correlated with the tumor diameter of PTC patients (P<0.001). The results of Li Linping et al. [14] showed that the number of patients with tumor focus diameter >1 cm (non thyroid microcarcinoma) was significantly higher than that without mutation. The average diameter of PTC tumor in BRAF V600E patients was 1.05 cm, while the average diameter of non-mutated tumor was 0.61 cm, which showed that the maximum diameter of BRAF V600E tumor is significantly larger than that of non-mutated tumor. In addition, some scholars think that the size of tumor diameter is one of the factors affecting the invasiveness of PTC. [9] At present, there are different opinions on this issue, which may need further study.

4.5. BRAF V600E gene mutation and distant metastasis

There are relatively few studies on BRAF V600E and distant metastasis. Some scholars believe that PTC has less distant metastasis, [9] and a few studies believe that they are related. For example, Wang Xuhong et al. [21] drew a conclusion that BRAF V600E is significantly related to distant metastasis in PTC patients (P=0.010). Other studies have shown that BRAF V600E has nothing to do with distant metastasis. Lai Yeqian [17] showed that distant metastasis was not related to BRAF V600E (P>0.05), but many PTC patients with distant metastasis could not be detected since the detection technology of distant metastasis is not mature and there is no relatively simple method of distant metastasis. At present, there is no accurate conclusion about the correlation between the two, and the results of many studies are contradictory. So, whether BRAF V600E mutation is indeed related to distant metastasis or not requires more researches and data to support.

4.6. BRAF V600E gene mutation and Hashimoto's thyroiditis (HT)

HT is the most common type of autoimmune thyroiditis and one of the most common causes of hypothyroidism. Its clinical characteristics are painless and diffuse thyroid lesions, and the titer of serum thyroid autoantibodies (TPOAb, TGAb, etc.) is significantly increased. [22] Chen Ni et al. [23] divided 158 PTC patients into HT group and non-HT group, with 79 cases in each group, and compared the invasiveness and BRAF V600E mutation of the two groups. They found the extrathyroidal invasion rate of HT group (5/18, 28%) was lower than that of non-HT group (13/18, 72%). It was considered that the combination of HT hindered the invasiveness of PTC to a certain extent, suggesting that the prognosis of PTC patients with HT was better. The mutation rate of BRAF V600E in non-HT group (72/79, 91.4%) was higher than that in HT group (63/79, 79.74%). Among 135 patients with BRAF V600E positive, the proportion of lymph node metastasis and extrathyroidal invasiveness in non-HT group were higher than that in HT group. Though there was no statistical

difference in lymph node metastasis between these two groups, it still suggested that BRAF V600E mutation in non-HT group may affect its prognosis. Yu Boyang et al. [24] studied 50 PTC patients with and without HT and concluded that PTC patients with HT had lower BRAF V600E rate, lower lymph node metastasis and extrathyroidal invasion than that without HT. By studying 210 patients with PTC and PTMC, Wang Chongjie et al. [25] found that the malignancy of PTC and PTMC with HT was lower than that without HT. HT had a certain restrictive effect on the lymph node metastasis of PTC and PTMC. Since the opinions reported in many studies are not unified, the relationship between BRAF V600E and HT is not clear. For example, Wang Xuhong et al. [21] proposed that BRAF V600E is significantly related to HT in PTC patients, while Wu wennian et al. [8] believe that BRAF V600E has no statistical significance with HT. However, a great number of researches have confirmed that PTC combined with HT reduces the lymph node metastasis, invasiveness and BRAF V600E mutation rate, suggesting that HT may be a potential protective factor in PTC patients. [9,23]

5. PTC molecular targeted therapy

Studies have shown that BRAF V600E is related to the initiation and progression of thyroid cancer. At present, many molecular targeted therapies targeting BRAF mutation and related pathways have achieved good results. [26] In the context of today's era of precision medicine, it is necessary to actively study molecular targeted drugs and make breakthroughs in the treatment of refractory papillary thyroid cancer. [27]

5.1. Sorafenib

Sorafenib (BAY43—9006) is the first Raf kinase inhibitor to enter clinical research. Sorafenib can not only inhibit Raf kinase activity, but also inhibit vascular endothelial growth factor receptor (VEGF1—3), platelet derived growth factor receptor β (PDGFR—β) and RET membrane receptor. Therefore, sorafenib can promote tumor apoptosis and inhibit angiogenesis at the same time. [26] Some experiments have shown that it can inhibit the growth and proliferation of KAT—5 cell line (papillary carcinoma cell line with BRAF mutation) and induce apoptosis. [28]

5.2. AZD6244

AZD6244 is a non-competitive inhibitor of selective MEK1 / 2, which shows its potential therapeutic prospect in other solid tumors. The results of phase I clinical trials of TC show its potential therapeutic effect, and it is easier to obtain disease remission with BRAF mutation. Although the overall treatment effect of AZD6244 is poor, the mPFS of TC patients with BRAF mutation is much higher than that of patients with wild-type BRAF gene (33 weeks and 11 weeks, respectively). Therefore, the selection of patients for this kind of drug treatment should be based on the BRAF gene type. [26]

5.3. Vemurafenib

In 2015, MD Anderson Cancer Research Center concluded through phase II clinical trial that Vemurafenib, a selective BRAF inhibitor, is a potentially effective and well tolerated treatment strategy for patients with advanced PTC carrying BRAF V600E mutations. [27]

6. Conclusion

In conclusion, BRAF V600E is the most common type of gene mutation in PTC, and it is also the most studied gene mutation related to PTC. The serine / threonine protein kinase encoded by BRAF gene is an important kinase in MAPK signal transduction pathway. After BRAF V600E gene mutation, it will continue to activate this pathway, enhance the ability of cell mitosis, and finally cause abnormal proliferation and produce tumor. BRAF V600E is also closely related to many clinicopathological characteristic of PTC, but the existing studies are controversial. Many research

results are contradictory. The main reasons for this phenomenon are as follows. First, the numbers of samples in some studies are small, and the results may not be universal. Second, some existing technologies are immature, and there may be missed detection. Third, many research samples come from one or more hospitals where the author is located. Whether different regions will lead to different physical fitness should be taken into account. Therefore, more and more in-depth research and analysis are still needed in the future. In addition, at present, some targeted drugs have been developed for this gene mutation, and the treatment effect is good. In the future, researchers should further study BRAF gene and its related biological structure, so as to better develop more targeted drugs and contribute to clinical treatment.

References

[1] Tallini G, Tuttle RM, Ghossein RA. The History of the Follicular Variant of Papillary Thyroid Carcinoma [J]. Clin Endocrinol Metab. 2017 Jan 1;102(1):15-22.

[2] Zhang Fuquan, Wang Xingyue, Gao Yingtang, et al. Research Progress on the relationship between BRAF gene V600E mutation and thyroid papillary carcinoma [J]. Shandong medicine, 2019,59 (34): 87-92

[3] Wang Weibin. The controversy of tumor clonal origin and the study of thyroid papillary carcinoma [D]. Department of medicine, Zhejiang University, 2010

[4] Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell.2014;159(3):676–690.

[5] Li Yalong. Treatment of 64 cases of thyroid papillary carcinoma [J]. Journal of clinical surgery, 2000,8(6):388-388.

[6] Cohen Y,Xing M,Mambo E,et a1.BRAF mutation in papillary thyroid carcinoma[J]. J Natl Cancer Inst,2003,95(8):625–627.

[7] Nikiforov YE. Molecular diagnostics of thyroid tumors[J].Archives Of Pathology & Laboratory Medicine,2011,135(5): 569.

[8] Wu Wennian, Bai Chao, Yang Zhenwei, Luo Jun. Correlation between BRAF ~ (V600E) gene and thyroid papillary carcinoma [J]. Xinjiang Medical Journal, 2021,51 (08): 877-881

[9] Zhu Guoquan, Ou Yangwei. Research progress of BRAF V600E mutation and thyroid papillary carcinoma [J]. Journal of molecular imaging, 2019,42 (1): 70-76

[10] Dong Linlin, Lu Yongliang, E Weijian, Zhang Xiang, Zhao Lingli. MAPK signaling pathway -- a new target for the treatment of hepatic echinococcosis [J]. Journal of clinical hepatobiliary diseases, 2022,38 (03): 714-718.

[11] Lin Tong, Sun Jianfang. BRAF protein and malignant melanoma [J]. Foreign medicine Dermatology and Venereology, 2004 (04): 247-249.

[12] Chen Shumin. Study on the correlation between thyroid micro papillary carcinoma and BRAF ~ (V600E) mutation and protein expression [D]. Fujian Medical University, 2015.

[13] Song Chuanwei. Expression of BRAF gene in thyroid carcinoma and its clinical correlation [J]. Contemporary medicine, 2018,24 (14): 76-78.

[14] Li Linping, Chai Fang, Wang Jun, Qu Lu, Wang Hongzhi, Lin Benrui, Liu Keyi, Huo Jianping, Zhu Zhansheng, Chai Dongqi, Cheng Mingming. Clinicopathological relationship between BRAFV600E gene mutation and thyroid papillary carcinoma [J]. Chinese Journal of Gerontology, 2020,40 (11): 2304-2306.

[15] Zang Xunxiong, Chen Jianwu, Lin Hongxian, Zheng Yan, Zhang Gaozhi. Study on BRAFV600E gene mutation in papillary thyroid carcinoma [J]. Modern practical medicine, 2009,21 (12): 1296-1297 + 1306 + 1270.

[16] Yu Jing, Wang Fengqin, Yao Wenjuan, Wu Dongmei, Zhang Weiqiang, Wang Qinzeng, Pang Siyi. Relationship between BRAFV600E gene mutation and central lymph node metastasis of thyroid papillary carcinoma [J]. Journal of Bengbu Medical College, 2020,45 (04): 493-496

[17] Lai Yeqian. Study on BRAF ~ (V600E) gene mutation in papillary thyroid carcinoma [J]. China modern drug application, 2017,11 (10): 31-33

[18] Wang Weina, Cao Yanzhen, Chen Haixia, Zhang Huan, Fang Xinzhi. Correlation between BRAF V600E gene mutation and clinicopathological features in papillary thyroid carcinoma [J]. Journal of Xinjiang Medical University, 2018,41 (01): 79-82 + 86

[19] Zhang Wei, Wang Nanpeng, Gao Qingjun, Zhou Yan, Duan Haisong, Zhang Wei, Zhao Daiwei. Relationship between BRAF ~ (V600E) mutation and clinicopathological features and thyroglobulin in papillary thyroid carcinoma [J]. Chinese Journal of basic and clinical medicine, 2015,22 (02): 200-203.

[20] Zhang Peng, Zhai Yuanpeng, Li Qian. Correlation between BRAF V600E mutation, ultrasonic features and cervical lymph node metastasis in papillary thyroid carcinoma [J]. Chinese Journal of practical diagnosis and treatment, 2020,34 (06): 611-614

[21] Wang Xuhong, Peng Chen, Li Le, Qin Yanchao. Relationship between BRAF V600E and tert promoter mutations and clinicopathological features of thyroid papillary carcinoma [J]. Chinese Journal of oncology, 2020,47 (11): 557-562.

[22] Li Fuyuan, Wang Shilong. Study on the relationship between BRAFV600E gene mutation and thyroid papillary carcinoma in Hashimoto thyroiditis [J]. Abstract of the world's latest medical information, 2016,16 (A4): 72-73.

[23] Chen Ni, Huang Xiaoling, Liu Liping, Li Maoping, Peng Xiaoqiong. Ultrasonographic features of thyroid papillary carcinoma with Hashimoto thyroiditis and its relationship with invasiveness and BRAF ~ (V600E) mutation [J]. Journal of clinical ultrasound medicine, 2020,22 (07): 515-518

[24] Yu Boyang, Ma Buyun, Zhou Jiehong, Tian Ye, Peng Yulan, BRAF gene expression and invasiveness in papillary thyroid carcinoma with Hashimoto's thyroiditis [J]. Progress in modern biomedicine, 2015,15 (15): 2833-2835 + 2828

[25] Wang Chongjie, Han Bing, Xing Haijun, Bai Yun, Zhu Shourong, Ji Buxin, Tao Yang. Effect of Hashimoto thyroiditis on lymph node metastasis and BRAFV600E mutation of thyroid papillary carcinoma [J]. Progress of modern general surgery in China, 2020,23 (08): 636-640.

[26] Dai Wenli, Cui Bangping, Hu Tao, et al. BRAF mutation and molecular targeted therapy in thyroid cancer [J]. Modern oncology, 2012,20(10):2177-2179.

[27] Yang Mengmeng, Chen Hongyue. Current situation of diagnosis and treatment of thyroid papillary carcinoma with traditional Chinese and Western medicine [J]. World latest medical information Abstracts (continuous electronic journal), 2020,20 (4): 41,45

[28] Kumar MS, Moore KE, Brose MS. Functional analysis of BRAF in a papillary thyroid cancer cell line [J]. Thyroid, 2004, 14: 712.