BRAF V600E突变对甲状腺乳头状癌发生及预后的影响

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［摘要］ 甲状腺乳头状癌(papillary thyroid cancer，PTC)是甲状腺癌最常见的病理类型。PTC通常预后良好，但近年来甲状腺癌的发病率逐年攀升。随着患病人数的不断增多，中晚期难治性甲状腺癌患者不再少见。因此，越发庞大的甲状腺癌患者群体的管理与诊治已成为巨大的考验。*BRAF* V600E基因突变是乳头状癌经典DNA相关标志物，目前已被广泛应用于甲状腺癌的术前诊断和预后评估，并且作为潜在的治疗靶点受到越来越多的关注。因此，正确全面的了解*BRAF* V600E基因突变可以帮助我们对PTC的发生、发展及生物学行为有更进一步的了解，并为PTC患者管理方式与治疗策略提供新的方向。

［关键词］ *BRAF* V600E突变；甲状腺乳头状癌；肿瘤发生；侵袭性；预后；生存分析

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Effects of *BRAF* V600E mutation on oncogenesis and prognosis in papillary thyroid cancer

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［**Abstract**］ Papillary thyroid cancer (PTC) is the most common pathological type of thyroid cancer, always with favorable prognosis. However, the incidence of thyroid cancer recently appeared to be an increasing trend. Because of the increased amount of patients, refractory thyroid cancer was not rare anymore. Hence, we are facing a big challenge how to manage and treat the increasing number of patients. *BRAF* V600E mutation is a classic DNA-relative biomarker for PTC, and widely used in preoperative diagnosis and evaluation of prognosis. As a potential therapeutic target, it attracted more and more attention. Recognizing *BRAF* V600E mutation can help us to know oncogenesis and biological behavior of PTC better and provide profitable treatment and management.

［**Key words**］ *BRAF* V600E mutation; Papillary thyroid cancer; Oncogenesis; Aggressiveness; Prognosis; Survival analysis

甲状腺乳头状癌(papillary thyroid cancer，PTC)是甲状腺癌中最常见的病理类型，占所有甲状腺癌的85%~90%。由于其惰性的生物学行为，PTC患者的预后良好，10年疾病总生存率甚至超过9.0%[1]。但近年来甲状腺癌的发病率逐渐攀升，中国的流行病学研究提示，近30年间甲状腺癌的发病率已升高近3倍，2015年中国新发甲状腺癌发病率达到90.0%[2]。此外，晚期难治性甲状腺癌的患者数量亦逐渐增多[3]。因此探寻甲状腺癌发生、发展的关键分子，既有利于甲状腺癌的早期诊断与合理治疗，亦有利于更好的理解常规治疗无效的原因，从而为新技术在晚期甲状腺癌中的应用奠定基础。

目前甲状腺癌相关标志物有许多，其中*BRAF* V600E基因突变是乳头状癌经典DNA相关标志物。随着分子诊断技术迅猛的发展，它已被广泛应用于甲状腺癌的术前诊断和预后评估。本文将针对乳头状癌*BRAF* V600E基因突变及其相关研究进展进行综述。

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1 PTC中*BRAF* V600E基因突变及相关通路

1.1 *BRAF* V600E基因结构及编码蛋白

*BRAF*又称鼠类肉瘤滤过性毒菌致癌同源体B1，与*ARAF*及*CRAF*具有同源性。该基因定位于人染色体7q34，相对分子质量为190×10^3，含18个外显子，有CR1、CR2和CR3共3个保守区域，含有7个转录区。该基因编码多种蛋白质，包括相对分子质量为94×10^3、含783个氨基酸残基的BRAF蛋白。该酶属于丝-苏氨酸蛋白激酶类，参与信号通路的转导[4]。

1.2 *BRAF* V600E基因突变及通路活化

*BRAF*基因是*RET*和*RAS*的下游信号分子，其编码的BRAF蛋白是RAS-RAF-MEK-ERK信号通路的关键要素，该信号通路调节细胞的生长、增殖和凋亡，当其发生变异后可能导致肿瘤的发生。

*BRAF*突变可发生于11和15外显子，但热点突变为*BRAF* T1799A点突变，即第1799位点的胸腺嘧啶被腺嘌呤替代，从而导致蛋白质产物中第600位的赖氨酸(V)被谷氨酸(E)替代(V600E，图1)。这一改变导致第599位的苏氨酸活化磷酸化位点附近插入了一个负性调节残基，从而影响了活化片段与结合ATP的P环的连接，从而致使*BRAF*的活化。该突变体可模拟活化区域的磷酸化过程，经级联式激活通过RAS-RAF-MEK-ERK信号传导通路引起细胞的异常增殖和分化，最终导致肿瘤的形成[5-6]。

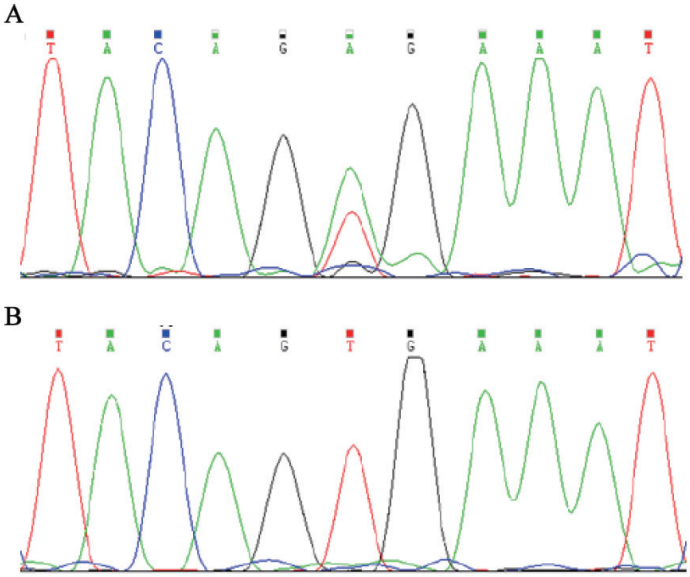

图 1 PTC中***BRAF*** T1799A突变(A)和野生型***BRAF***基因(B)

Fig. 1 ***BRAF*** T1799A mutation in PTC (A) and wild type ***BRAF*** gene (B)

2 *BRAF* V600E突变在甲状腺结节术前分子诊断中的作用

2015年美国甲状腺协会发布的甲状腺结节术前分子诊断共识[7]指出，*BRAF* V600E突变对于PTC的诊断、预后及选择合适的治疗方案均具有关键作用。术前穿刺基因诊断可用于甲状腺结节良恶性的鉴别。*BRAF* V600E突变常用的检测技术包括聚合酶链反应、桑格测序法和焦磷酸测序法等。由于术前细胞学穿刺组织稀少，因此目的基因的检测结果会因组织的获取量多少存在异质性。术前分子检测的出现有效地提高了甲状腺癌的检出率，但分子检测的应用需考虑费用及诊治不足带来的不良后果等因素。目前，*BRAF* V600E突变检测多应用于甲状腺结节术前细针穿刺细胞学检查(fine-needle aspiration biopsy，FNAB)标本。但有文献报道，单纯的术前FNAB对于甲状腺癌诊断的假阴性率低于3%[8]，因此绝大部分患者此时并不需进行分子检测，只有单纯FNAB无法明确诊断或高度怀疑恶性的患者才需要进行。此外，由于*BRAF* V600E突变与许多临床病理学因素相关，基因诊断在对患者病情全面术前评估中的作用

正在探索中。

3 PTC中*BRAF* V600E基因突变概率及其与临床病理因素和预后的关系

3.1 PTC中*BRAF* V600E突变情况

据文献报道，*BRAF* V600E突变在所有恶性实体肿瘤中发生的概率为7%~9%，其中恶性黑色素瘤中的突变概率最高，接近60%[9-13]。PTC被发现为*BRAF* V600E突变比例较高的实体性肿瘤。由于各个单中心报道中所采用的检测手段、测序方法和组织获取等因素的差异，*BRAF* V600E突变概率不尽相同。*BRAF* V600E突变概率为31.3%~86.1%[14-15]。各研究报道的突变概率总结见图2。近年来全球范围内甲状腺癌发病率呈现不断上升的趋势，这其中PTC新发病例的增多占主要原因[16-17]。其中超过50%的乳头状癌为微小乳头状癌(papillary thyroid micro-carcinoma，PTMC)，因此如何更好的处理已有且不断增多的PTMC患者成为甲状腺专科医师面临的突出问题。这其中如何理解PTMC的生物学行为起到关键作用。尽管在所有PTC患者中，*BRAF* V600E基因突变的平均概率达到40%，但在PTMC患者中，其突变概率明显偏低[18]，甚至有报道指出，在小于5 mm的PTMC患者中，*BRAF* V600E基因突变者仅为18%[19-22]。因此对于PTMC患者，*BRAF* V600E基因突变的识别更有利于危险度分层及个体化治疗方案的选择。

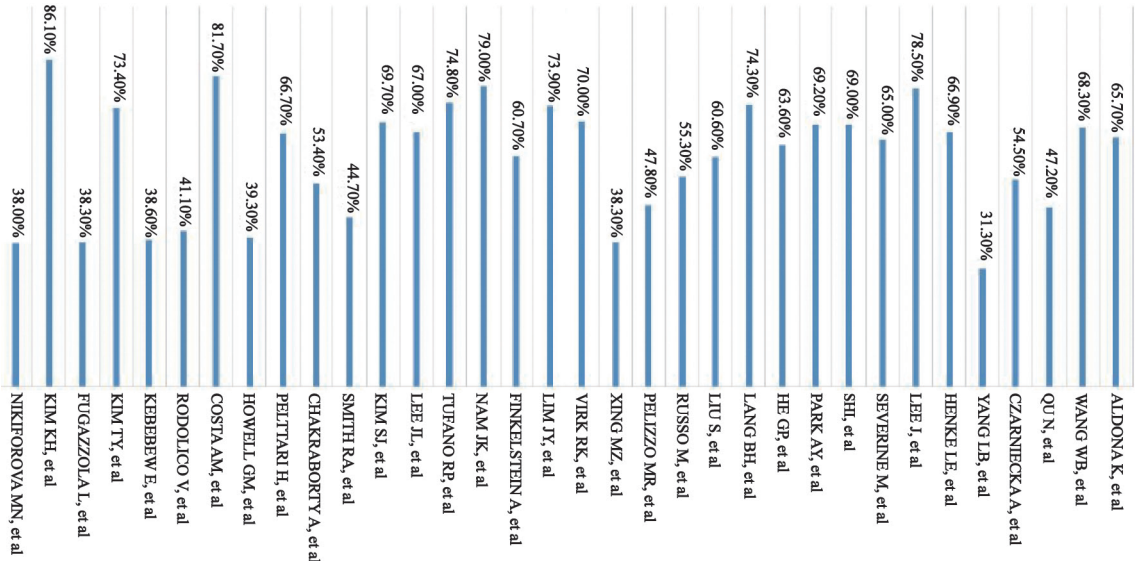

图 2 *BRAF* V600E突变率情况

Fig. 2 *BRAF* V600E mutation rate

3.2 PTC中*BRAF* V600E突变与临床病理学因素关系研究

众多国内外文献相继报道了PTC中*BRAF* V600E突变与不良临床病理学因素之间的关系，如疾病晚期、侵袭性特征等，而这些特征已作为PTC危险分层的关键因素被越来越多的评价系统所接纳[23-27]。一系列文献提示，*BRAF* V600E突变与患者高龄、原发肿瘤较大、经典PTC亚型、原发肿瘤腺外侵犯、多灶性、双侧病灶、初治时局部淋巴结转移、远处转移和TNM高分期等不良因素密切相关[28-33]。*BRAF* V600E突变与患者性别之间的关联，目前仍有争论[15,32,34-36]。此外，Smith等[32]和Lim等[35]的研究均指出，*BRAF* V600E突变与合并桥本氏甲状腺炎呈显著负相关，这提示淋巴细胞浸润性炎症与PTC的发生有密切关联。Qu等[9]的一项回顾性研究首次指出，*BRAF* V600E突变与高体重指数(body mass index，BMI)密切相关，当BMI超过24.9 kg/m^{2}时，*BRAF* V600E突变风险增加约7.6倍(OR=7.645，95%CI：1.275~45.831)。

BRAF V600E在微小肿瘤中的突变概率偏

低，而Virk等[10]及Rodolico等[18]的2项针对PTMC患者的回顾性研究均指出，*BRAF* V600E突变是区域淋巴结转移的独立预测因素；Lang等[12]在一项纳入845例原发灶小于2 cm的PTC患者的研究中指出，单因素分析提示，*BRAF* V600E突变与肿瘤大小、原发肿瘤腺外侵犯、淋巴结转移数目、单侧Ⅵ区淋巴结转移数目及Ⅵ区淋巴结转移比例呈正相关(P均<0.001)；而校正后的多因素研究提示，*BRAF* V600E突变仅与中央区淋巴结转移(OR=1.647，95%CI：1.101~2.463)呈显著正相关。Pelttari等[34]的研究关注51例TNM Ⅰ、Ⅱ期的PTC患者，结果显示，*BRAF* V600E突变与性别、年龄、肿瘤大小、腺外侵犯和区域淋巴结转移均无显著相关性(P>0.05)。因此，*BRAF* V600E突变在PTMC或早期PTC中的危险预测作用仍需进一步探索。

3.3 PTC中*BRAF* V600E突变与疾病预后的关系

许多研究认为，*BRAF* V600E突变与疾病不良预后有关[11,30-31,37]。例如Howell等[11]、Kebebew等[30]、Costa等[31]和Xing等[37]的研究均指出，*BRAF* V600E突变是PTC复发的独立危险因素；Russo等[38]在研究中指出，生存风险单因素分析结果提示，*BRAF* V600E突变与肿瘤复发(HR=3.5，95%CI：1.2~10.3)呈正相关，但多因素结果提示，其与肿瘤复发(HR=2.8，95%CI：0.7~11.8)无显著相关性。此外，Fugazzola等[13]、Kim等[15]的研究亦显示，*BRAF* V600E突变与肿瘤复发无显著相关性。这与各项研究纳入病例数、患者特征及随访时间等因素均有关联。

近期研究提示，在*BRAF* V600E突变且治疗后复发肿瘤中，碘代谢机制被下调，导致不良预后。可能机制为*BRAF* V600E突变致MAPK途径下游基因持续激活，引起钠-碘同向转运体(sodium iodide symporter，NIS)的表达稍下降，而NIS的定位异常更为明显，*BRAF*野生型NIS定位于甲状腺滤泡膜，而*BRAF* V600E者NIS未能精确定位于细胞膜，更多地弥散分布于细胞质中[36]。另外，垂体瘤转化基因结合因子及转化生长因子β表达增加[39]、TSH受体启动子甲基化等都与*BRAF* V600E突变致摄碘下降有关。

由PTC引发的肿瘤特异性死亡较为少见。Niederer-Wüst等[40]在回顾性单因素生存分析中发现，*BRAF* V600E突变与总生存率无显著相关(HR=0.46，95%CI：0.14~1.49)，且与肿瘤特异死亡亦无显著相关性。另外几项回顾性研究的结果类似[31,41]，但考虑到*BRAF* V600E突变与肿瘤复发的关联，对于晚期*BRAF* V600E突变的PTC患者需给于积极治疗以改善预后。

4 结语

综上所述，*BRAF* V600E突变与PTC的发生、发展及生物学行为存在密切的联系。通过检测患者*BRAF* V600E突变状态，不仅可以提高术前确诊率，同时可指导PTC的治疗及预后预测。然而，在PTC患者中，*BRAF* V600E突变检测的应用指征及具体实行策略还需进一步的明确。正确评价*BRAF* V600E突变与临床病理及预后的关系，可为表达*BRAF* V600E突变的晚期PTC患者提供新的治疗思路。*BRAF* V600E突变作为PTC的重要分子标志物，会对其未来的诊疗产生深远的影响。

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