Impact of different methods on the detection frequency of BRAF mutation in Papillary Thyroid Carcinoma (PTC)

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INTRODUCTION

Still there is a discussion about the importance of diagnostic and prognostic relevance of V600E mutation in the BRAF gene. The wide range of BRAF V600E mutation frequency (40% to 80%) does not facilitate this task. Population diversity and genotyping methodology are suspected sources of differences seen in the mutation frequencies detected by different studies. The aim of this study was to assess the prevalence of mutations in the BRAF V600E, depending on the diagnostic method used (direct sequencing and ASA-PCR) analyzed on a Holy cross population.

MATERIALS AND METHODS

Tumors from 411 consecutive patients with PTC genotyped for BRAF V600E using capillary sequencing and allele specific amplification PCR (ASA-PCR). DNA subjected to genotyping isolated following macrodissection using tissue area marked by the pathologist. The level of sensitivity of the capillary sequencing is generally estimated at 10-20% of mutated allele in WT alleles and the sensitivity of the ASA-PCR technique is about 1% of mutated allele in WT alleles.

RESULTS

Of the 411 cases genotyped using both methods capillary sequencing and ASA-PCR BRAF V600E mutation detected in 270 cases (65.7%) Fig. 1A, 2 i 3A. Additionally detected 8 (1.9%) other mutations in the BRAF near codon V600 Fig. 3A. In total, this gives 278 (67.6%) cases with mutation Fig. 3A. In contrast, no mutations were found in 121 (29.4%) cases Fig. 1B i 3A. Because of DNA degradation we failed to get a result in 12 (2.9%) cases Fig. 3A. If we take into account only the results obtained by sequencing, BRAF V600E mutation detected in 186 (45.3%) cases and 8 (1.9%) mutations in the vicinity of codon BRAF V600E Fig. 3B. In total sequencing detected 194 (47.2%) mutations in the BRAF Fig. 3B. In 151 (36.7%) cases no mutation detected Fig. 1B i 3B. In 31 (7.5%) cases sequencing result was inconclusive Fig. 1C i 3B. DNA degraded in 35 (8.5%) cases and we didn’t obtained the result Fig. 3B. However, with the ASA-PCR method BRAF V600E mutations were found in 270 (65.7%) cases Fig. 2. This method only detects the V600E mutation, so it hasn’t detected eight cases with mutations located in the vicinity of the BRAF V600 codon. In 27 (6.6%) cases the DNA was degraded, and one result was inconclusive Fig. 3C.

CONCLUSIONS

The frequency of detected mutations in the BRAF depends on the method. The combination of methods with different sensitivity and spectrum of detected mutations increases the incidence of PTC with the presence of mutations in BRAF. Differences in the frequency of BRAF mutations in PTC described in the literature may be the result of applied research methodology. This might be attributed to the tumor clonality phenomenon. It is recommended to use a combination of methods in the diagnosis of mutations in the BRAF PTC.