

ABSTRACT

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BRAF mutations in metastatic malignant melanoma.

Diploma thesis

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Background: Melanoma is malignant disease with increasing incidence. Treatment of advanced stage of melanoma is still limited. With a progress of knowledge in genetics and tumorigenesis, the incidence of mutated BRAF protein was observed at 50 % of melanomas. In 80-90 % mutated melanomas contain *BRAF V600E* mutation. The aim of study was to establish a suitable molecular biological method for the diagnosis of mutations in codon V600 BRAF.

Methods: Cobas 4800 BRAF V600 mutation test and BRAF StripAssay test were used to analyze DNA. Cobas 4800 BRAF V600 mutation test is based on PCR using TaqMan probes designed for the wild-type and mutant *BRAF V600E* sequence. BRAF StripAssay test is based on PCR amplification with biotinylated primers and subsequent hybridization of the stripped with allele-specific oligonucleotide probes. Examined DNA samples were derived from 35 patients with advanced malignant melanoma or from archive of laboratory.

Results: *BRAF V600* mutation was detected in approximately half of the tumors, consistent with the results of other studies. In comparison methods Cobas test went better. Part of the samples was by Cobas 4800 BRAF V600 test positive, but by BRAF STRIPASSAY was detected as *BRAF V600E* negative. This suggests that the samples could carry *V600K* and *BRAF V600D* mutation or insufficient sensitivity BRAF STRIPASSAY test. In two metastases were detected mutations, although the primary tumor was negative (wild-type).

Conclusions: The cobas 4800 BRAF V600 showed greater sensitivity and a wider range of captured mutation compared with BRAF StripAssay. Different quality or sensitivity of the test is diagnostically significant. Results indicate that Cobas 4800 BRAF V600 test could be used to diagnose almost 100% of mutated BRAF melanomas. The detection of mutations correspond literature. The results show the importance of testing real-time sample pre-treatment, metastases can not rely on the results of the examination of the tumor.