

Evaluation of BRAF (V600E) Mutation by Pyrosequencing Assay

utmb Health

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Introduction

BRAF is a human gene that encodes B-Raf protein. B-Raf protein is more formally known as serine/threonine-protein kinase B-Raf which is involved in the MAP kinase/ERK-signaling pathway. Activation of BRAF leads to successive phosphorylation and activation of two other kinases (MEK/ERK), ultimately allowing ERK to enter into the nucleus of the cell and regulate gene expression. Mutations in BRAF are thought to contribute to oncogenesis by inhibiting apoptotic pathways and promoting melanocytic proliferation and has emerged as an important biological marker for diagnosis, prognosis and therapeutic guidance for human cancers (Kolch, 2000; Erhardt, Schremser & Cooper, 1999; Pollock et al., 2002; Sharma & Gulley, 2012).

BRAF is clinically relevant for diagnosis, prognosis, and treatment of oncology patients. With the development of anti-mutant inhibitors, accurate detection and interpretations of BRAF mutation status is becoming more important. BRAF mutation testing is available at University of Texas Medical Branch (UTMB) to aid in clinical management of oncology patients, but BRAF mutation status in patients at UTMB and test performance are unknown. The purpose of this study was to evaluate the BRAF mutation status in patients at UTMB. BRAF mutation testing performance was also evaluated by comparing overall frequency of BRAF mutations in patients at UTMB with the previous studies.

Methods

This retrospective, cross sectional study was approved by UTMB's Institutional review board (IRB)

176 samples were tested at the MDL using Pyromark pyro sequencing technology from Qiagen (Qiagen, 2014) which is programmed to cover hotspots of BRAF gene.

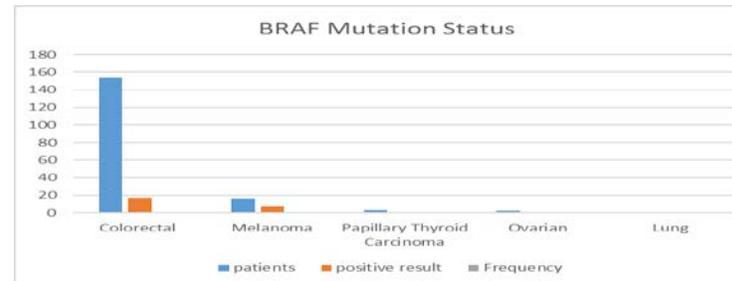
All samples showing more than 10% mutant alleles were reported as positive for BRAF mutation

Patient's age, sex, ethnicity, type of cancer, tumor type (primary or metastatic) were analyzed in this study

Pearson's chi-squared test was used with a significance level of 0.05 to determine if any differences among groups are statistically significant. The Student's t-test was used to for the analysis of age differences.

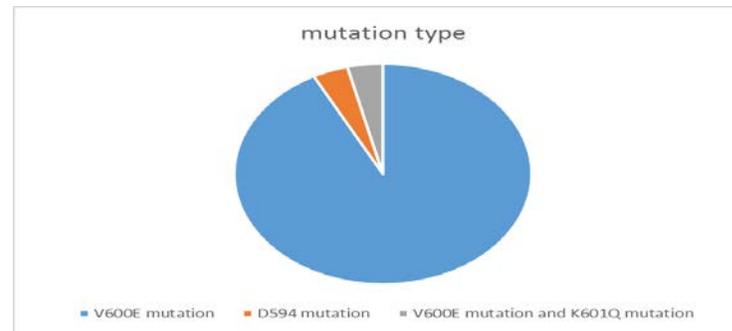
Results

A total of 176 patients who were tested for BRAF mutation between September 2011 and June 2015 had one of the following diagnoses; colorectal cancer, melanoma, papillary thyroid carcinoma, ovarian cancer and lung cancer. 27 BRAF mutation were found from 176 patients, the overall frequency of BRAF mutation was 15% (27/176), in which 11%, 44% and 33% were found, respectively in colorectal cancer, melanoma, papillary thyroid carcinoma. Table 1 lists the mutation frequency for each diagnosis.



Results

Specific subtypes of BRAF mutations: The most prevalent mutation found in this study was V600E mutation, accounted for a large percentage of the total BRAF mutations found (96%). One patient had a D594 mutation. One patient had both the V600E mutation and a K601Q mutation.



Results

Expected frequency of BRAF mutation in colorectal cancer was 12%, the weighted average calculated from the previous publications. The expected range was 5-23%. The frequency in colorectal cancer patients at UTMB was 11%. The expected frequency of BRAF mutation in melanoma was 45%. The expected range was 29-68%. The frequency in patients at UTMB was 44%. There was no statistically significant between frequency of BRAF mutation in colorectal cancer and melanoma at UTMB and calculated frequency of BRAF mutation from the previous publications.

There was a trend for higher mutation frequency in female patients, which was significant both overall ($p=0.017$) and in the colorectal cancer group; 25% of females ($n=41$) compared to 7% ($n=116$) of males ($p=0.009$) with colorectal cancer harbored the mutation. In the melanoma group, 75% of females ($n=4$) had the mutation compared to 33% of males ($n=12$). All male patients with PTC harbored the mutation while 0% of females harbored the mutation, however, this association did not reach significance ($n=1$ and $n=3$, respectively).

Ethnicity was known for 153 patients. In the colorectal cancer group, Caucasians were found to have the highest mutation frequency at 14% ($n=84$), followed by African Americans at 9% ($n=34$). The Hispanic population had the lowest rate at 4% ($n=24$). The difference did not reach significance

Conclusions

The most prevalent mutation found in this study was V600E mutation, accounted for a large percentage of the total BRAF mutations found (96%).

The results of this study showed that BRAF mutations found when tested at UTMB MDL were comparable with the published literature. Total BRAF mutations found in this study were consistent with other published studies.

This study indicates that the population at UTMB is not substantially different from those previously tested. This also supports the Pyrosequencing Assay is a reliable Pyrosequencing Assay

References

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