NATIONAL CANCER INSTITUTE

NOVARTIS

Detection of Mutations in the Mitogen-Activated Protein Kinase Pathway (MAPK) in Human Melanoma

Jolita Gibavičienė[1], Justina Gaiževska[1,2], Otilija Kutanovaitė[1], Linas Senkus[1], Rasa Sabaliauskaitė[1], Sonata Jarmalaitė[1,2]

[1]National Cancer Institute, Vilnius, Lithuania; [2]Division of Human Genome Research Laboratory, Life Sciences Center, Institute of Biosciences, Vilnius University, Vilnius, Lithuania

Introduction & Objectives

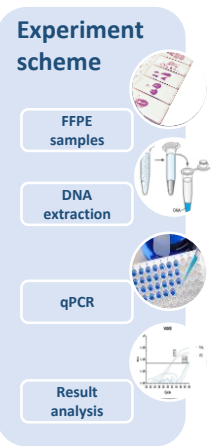
In melanoma, one of the most commonly activated signaling pathway is MAPK, which over-activation leads to increased cell proliferation and promotes disease progression. Abnormal activation is typically induced by oncogenic mutations, usually in *BRAF* and *NRAS* genes. Although genetic alterations in both genes can be detected in approximately 40 and 20% of cases respectively, the association between lymph node metastasis and presence of *BRAF* mutations in patients with melanoma remains uncertain. What is more, in addition to known alterations in *BRAF* and *NRAS*, it is known that 4-9% of melanomas harbor non-silent mutation, C > T transition, that results in a p.Pro29Ser substitution in *RAC1* gene. The identification of statistically significant hot spot mutations in *BRAF*, *NRAS* and *RAC1* genes, evaluation of *BRAF* and *NRAS* concurrency in primary tumor and sentinel nodes, offers more genomic evidence in mechanism of this malignancy, hence needs to be elucidated.

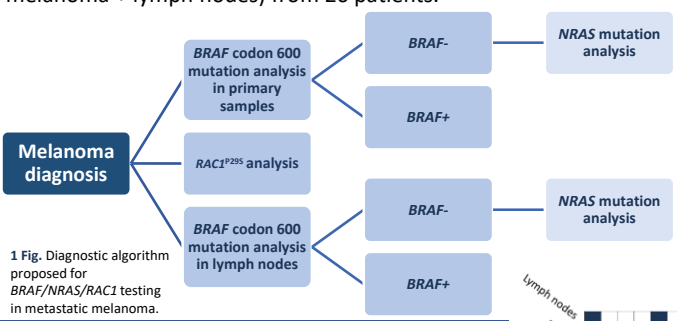
The aim of the study was to clarify the incidence of *BRAF*, *NRAS* and *RAC1* mutations and compare manifestation of *BRAF* and *NRAS* alterations in paired samples of lymphatic metastases and primary melanoma.

Materials & Methods

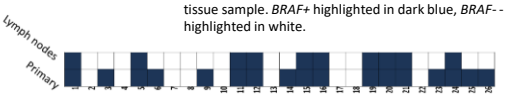
BRAF, *NRAS* and *RAC1* mutations were assessed using real-time PCR instrument, IVD kit and TaqMan genotyping assay. Overall, detection of *BRAF* mutation status was performed in 52 formalin-fixed, paraffin-embedded paired samples (primary melanoma + lymph nodes) from 26 patients.

11 more patients were tested for *BRAF* mutations only in primary melanoma (n=37). *NRAS* alterations were determined for *BRAF* negative patients only. *RAC1* status was determined in 18 patients.





1 Fig. Diagnostic algorithm proposed for *BRAF/NRAS/RAC1* testing in metastatic melanoma.

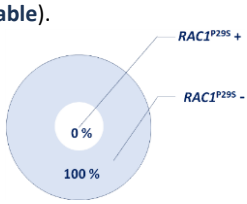


2 Fig. Distribution of *BRAF* mutations in individual patients primary melanoma sample and lymph node tissue sample. *BRAF+* highlighted in dark blue, *BRAF-* - highlighted in white.

Results

In this study, we detected that **57%** of all tested patients (21/37) carried a *BRAF* mutation in primary melanoma samples, including *BRAF* V600E (7/21) and *BRAF* V600M (1/21). Multiple *BRAF* mutations (V600E/R, V600E/K/R, V600E/K/R/M) were found in one patient (**1 Table**).

Mutation	Number of patients with *BRAF/NRAS* mutation in primary melanoma samples (n, %)	Number of patients with *BRAF/NRAS* mutation in lymph node samples (n, %)
BRAF	**21**	**10**
V600E	7 (33%)	7 (70%)
V600M	1 (5%)	-
V600E/R	1 (5%)	1 (10%)
V600E/K/R	10 (47%)	-
V600K/R/M	-	1 (10%)
V600E/K/R/M	2 (10%)	1 (10%)
NRAS	**4**	**4**
G12D	-	2 (50%)
G12C	1 (25%)	-
Q61K	-	1 (25%)
Q61L	1 (25%)	-
Q61R	2 (50%)	1 (25%)



3 Fig. *RAC1*^{P29S} mutation status in analyzed primary melanoma samples.

1 Table. *BRAF* and *NRAS* mutations detected in primary melanoma and lymph node tissue samples.

In more than one-third (10/26) of *BRAF* positive patients, good concordance in *BRAF* mutation status was found between the primary tumour and lymph node tissue samples. Discordance in *BRAF* mutation status was found in seven patients whom lymph node metastases were sampled (**2 Fig.**). We identified 4 *NRAS* mutations in primary melanoma samples (n=10): G12C (1/4), Q61L (1/4), Q61R (2/4) and another 4 in lymph node samples (G12D (2/4), Q61K (1/4), Q61R (1/4)) (**1 Table**), 3 of which were detected in different than first four mutations patients. All 18 patients, that were tested for highly recurrent *RAC1*^{P29S} mutation, were *RAC1*^{P29S} negative (**3 Fig.**).

Conclusion

Our study identified frequency of *BRAF* and *NRAS* mutations in melanoma patients. We did not find any melanoma cases with *RAC1*^{P29S} mutation in this study. We also determined that the association between lymph node metastasis and presence of the *BRAF* mutations in primary tumour sample, requires further investigation.