Molecular genetic testing for BRAF V600 gene mutations in melanoma in the Republic of Kazakhstan

Relevance: Along with other diagnostic methods, all current recommendations and protocols for malignant tumors' diagnostics and treatment require mandatory molecular genetic testing at the initial diagnosis and in case of disease progression. The discovery of the BRAF gene mutation in skin melanoma allows the treatment with tyrosine kinase inhibitors, so-called BRAF inhibitors. This increases the probability of tumor response by half and opens up new therapeutic options.

The study aimed to identify the BRAF V600E gene mutation frequency in patients with stage III-IV melanoma in the Republic of Kazakhstan to determine the need for targeted therapy.

Results: 2251 persons are currently registered with melanoma in the Republic of Kazakhstan, with 355 new primary melanoma cases in 2019. Of them, 54% were diagnosed at stages III-IV and were subject to molecular genetic testing. 278 of 2251 patients were sent for BRAF mutations testing. The mutations were detected in 105 (37.7%) patients.

Conclusion: The introduction of molecular genetic testing for malignant neoplasms under the Comprehensive Cancer Control Plan framework for 2018-2022 will increase the use of molecular genetic methods in the Republic of Kazakhstan. Molecular genetic testing is an important step in diagnosing melanoma and choosing the appropriate therapy to personalize cancer treatment.

Keywords: melanoma, molecular genetic testing (MGT), BRAF V600E gene mutations, personalized approach, targeted therapy.

Introduction: Melanoma incidence is steadily growing in different countries, including Kazakhstan (RK). Over the previous decade, skin melanoma has grown by 63% in the RK territory [1]. According to the World Health Organization, melanoma incidence will add a quarter over the next decade. Today, melanoma ranks first among all solid tumors after lung cancer by the incidence growth rate [2]. According to the World Health Organization, melanoma incidence in the Republic of Kazakhstan in 2019 was 16.4 per 100,000 people, which is 2.5 times higher than in 2010. The melanoma incidence is steadily growing in different countries, including Kazakhstan (RK). Over the previous decade, skin melanoma has grown by 63% in the RK territory [1]. According to the World Health Organization, melanoma incidence will add a quarter over the next decade. Today, melanoma ranks first among all solid tumors after lung cancer by the incidence growth rate [2].

In such an overactive state, the “broken” BRAF protein uncontrolled transmits signals to the cell nucleus that triggers continuous cell division. The BRAF gene mutations found in more than 50% of melanoma cases is a reason for the BRAF protein constant autonomous activity [3].

The achievements in molecular biology allowed deciphering a complex genetic profile of melanoma, prove its heterogeneity, and identify possible molecular targets for targeted therapy [4]. Mitogen-activated protein kinase and, in particular, the ERK signaling cascade has become the subject of intensive research in melanoma. The signal from the receptor on the cell surface through the RAS, RAF, MEK and ERK proteins is transmitted to the cell nucleus through this transduction cascade, thus regulating the cell growth, differentiation, and apoptosis (Figure 1) [5]. This cascade activation can result from the involvement of receptors (growth factors located on the tumor cell surface) and the mutations in the RAS and RAF family genes involved in the cell growth regulation. Mutated RAS proteins lose the ability to hydrolyze the guanosine triphosphate bound to them into guanosine diphosphate and lose the negative autoregulation mechanism [5]. The existing disorders lead to permanent activation of the RAS/RAF/MEK/ERK signaling cascade and, consequently, malignant transformation of cells. The RAF kinase family includes three proteins: RAF, BRAF, and CRAF. BRAF protein kinase is a component of the RAS-RAF cascade [5, 6]. BRAF oncogenic mutations in melanoma are quite frequent, 40 to 60% [3]. In most cases, the NRAS and BRAF mutations are not present in the same melanoma, so we can assume their functional redundancy. About 80% of BRAF mutations are V600E, less often V600K mutations are observed (5-30%), and most rarely - V600R, V600D [7, 8]. In BRAF gene mutations (for example, V600E mutation - replacement of valine with glutamine), the kinase activity of the BRAF enzyme increases concerning the subsequent MEK protein kinase transduction in the intracellular signaling pathway. This results in constant activation of the RAS-RAF-MEK-ERK signaling cascade, uncontrolled proliferation, and blockage of apoptosis.

In such an overactive state, the “broken” BRAF protein uncontrollably transmits signals to the cell nucleus that trigger continuous cell division. The BRAF gene mutations in the late stages of melanoma are associated with a poor prognosis and a more aggressive course of the disease [10].

For decades, there were no other treatment options for melanoma other than chemotherapy with dacarbazine, with an extremely short-term (approximately 5-6 months) response rate of less than 10%. The median life expectancy of patients did not exceed a year. With the advent of targeted therapy, the patients whose tumor contains BRAF mutations can receive a BRAF inhibitor with the response probability of about 50% [11]. Regardless of a BRAF mutation, they can receive an immune checkpoint inhibitor, which delivers a long-term (more than five years) response to treatment of about 20% [12, 13].
Figure 1 – General map of signaling pathways involved in apoptosis. The role of Raf kinases (such as B-Raf) is indicated in the center of the diagram [9]

All modern recommendations and protocols for the diagnostics and treatment of malignant neoplasms, along with other diagnostic methods, require mandatory molecular genetic testing (MGT) both during the initial diagnostics and in the case of disease progression [14]. The determination of tumor biomarkers and the body genetic stability is of greatest importance in the light of the tactics for prescription of targeted drugs, in connection with the constant introduction of new molecules for the treatment of malignant neoplasms, as well as the widespread use of the so-called tissue agnostic approach, when the prescription of a certain group of drugs takes into account not only and not so much the localization and type of tumor but the identification of clinically significant mutations and biomarkers [14].

The study aimed to identify the BRAF V600E gene mutation frequency in patients with stage III-IV melanoma in the Republic of Kazakhstan to determine the need for targeted therapy.

Materials and methods: The study used statistical data on melanoma incidence in the Republic of Kazakhstan collected by the Kazakh Institute of Oncology and Radiology. The number of patients was calculated based on the data from the information system for registration of cancer patients (IS “Electronic Register of Cancer Patients”) and taking into account the statistical indicators of the RK cancer service [15]. Leading oncological associations recommend MGT of the tumor for BRAF mutations starting from stage III cancer. Statistical data was adjusted for the availability of biological material during diagnostic actions or surgery. The material for MGT was the tumor tissue cells obtained from paraffin blocks containing postoperative and biopsy material of patients with melanoma, first of all, in its advanced or metastatic forms. DNA from the formalin-fixed and paraffin-embedded tumor was extracted by liquid phase method using the cobas® DNA Sample Preparation Kit by Roche Diagnostics following the manufacturer’s instructions. DNA amplification by PCR in real-time mode to detect BRAF mutations was performed using the cobas® 4800 BRAF V600 Mutation Test reagent kits by Roche Diagnostics.

Results and discussion: In the examined group of patients, men accounted for 39.6% vs. 60.4% women. The age composition of the examined patients: 15.4% were below 40 years, 41.4% were aged 40 to 60 years, and 43.2% were above 60 years. The average age was 56.5 years.

The highest mutation frequency was detected in Shymkent, South Kazakhstan region (64%), Kokshetau, Akmola region (60%), Pavlodar, Pavlodar region (55%), and Turkistan (50%) (Figure 2). The cohorts by region differed in size; some of them were too small to reveal significant differences in mutation frequencies. Most patients were referred for BRAF testing from Qostanai (n=35) and Karaganda (n=27) regions and the city of Almaty (n=31).

In total, in 2019, 2,251 people were registered with melanoma in the RK. Of them, 355 were initially diagnosed with melanoma. 54 (15.2%) of initially diagnosed patients had stage III-IV cancer and were subject to MGT. In fact, only 278 patients were referred for BRAF testing, while mutations were detected in 105 (37.7%) patients (Figure 3). As can be seen from the results of the conducted MGT, BRAF testing should be planned based on the number of registered patients instead of the number of initially diagnosed patients since, in this case, the need for testing is much higher.
Figure 2 – The patients examined and the share of the BRAF V600 mutations detected in melanoma in the RK

The study was made possible thanks to the Comprehensive Plan to Combat Cancer Diseases for 2018-2022, which was approved by the Decree of the Government of the Republic of Kazakhstan dated June 29, 2018, No. 395 “On Approval of the Comprehensive Plan to Combat Cancer Diseases in the Republic of Kazakhstan for 2018-2022” and is being implemented in the Republic of Kazakhstan since 2018. Since the Comprehensive Plan provides for MGT in various tumors, including skin melanoma, MGT is provided free of charge, at the expense of allocated funding, to all patients diagnosed with melanoma, colorectal cancer, or lung cancer.

The data was obtained from the reference centers, including the laboratories of the Kazakh Institute of Oncology and Radiology (KazIOR, Almaty), the Multidisciplinary Medical Center (Nur-Sultan), and the Regional Oncological Dispensary (Karaganda). The choice of the reference centers for mass conduct of MGT was determined by the presence of specialized laboratories conducting pathomorphological, immunohistochemical, and MGT studies and trained specialists. In the coming years, the list of laboratories shall be expanded to simplify the delivery of biological samples and speed up the examination process. KazIOR has proposed adhering to an MGT algorithm based on international recommendations to stratify patients with melanoma. Following these recommendations, all patients diagnosed with stage III-IV melanoma are subject to mandatory MGT [14, 16, 17].

Thus, the study of pathogenetic foundations of melanoma development has significantly advanced, and a huge step was made to identify new potential molecular targets. The BRAF molecular target discovery and understanding of its role in melanoma pathogenesis have formed the basis for developing a new class of drugs – low-molecular-weight BRAF inhibitors. These drugs block the pathologically activated signaling cascade triggered by the BRAF V600 mutation and inhibit tumor development. However, the impact of this drug group on tumor cells with a normal BRAF sequence may cause a pathological activation of the RAS-RAF-MEK-ERK cascade and provoke melanoma growth. This phenomenon evidences the exceptional importance of reliable BRAF gene status diagnostics [18].
The identified gene mutations associated with sensitivity to inhibitors require targeted therapy in the first line. A repeated MGT might be required to select adequate therapy at progressive disease due to a possible tumor progression and clonal evolution or emergence of new clinically significant mutations and resistance mutations.

Conclusions: Thus, MGT in melanoma is an integral part of diagnostics and therapy selection to personalize cancer treatment. The mutations were found in 105 (37.7%) of 278 patients with melanoma sent for BRAF testing from all regions of the RK. BRAF Test Planning should not be based on the number of initially diagnosed patients but the number of registered patients. The introduction of MGT for malignant neoplasms under the Comprehensive Plan is the basis for the widespread use of molecular genetic methods to diagnose and personalize cancer treatment in the RK.

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Казахстан Республикасының меланома кезінде БРАФ генінің V600 мутациясының молекулярлық-генетикалық тестиңе синағы

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Актуальность: Меланома, ерекшеленген сарпакқа қарағанда, өзінің жұлдыз құрмалық қасиеттері мен мұнай-ге нәсілдетілі түсіндірілген адамдарда болады. Меланомадан халықтың батысының 3%-і, және оның 4%-ға дейін жұлдыз құрмалық қасиетті болуы мүмкін. Бұл болуы мүмкін, ол майлы, тамақтық, сақтау құралдарының қолданылуы қажет етеді.

Цель исследования: Определение количества первично выявленных пациентов с меланомой в РК на 2018-2022 годы.

Результаты: Общее количество первично выявленных пациентов с меланомой в РК в 2019г составило 355 человек. Из них 54% составили пациенты с III и IV стадиями, то есть пациенты, подлежащие молекулярно-генетическому тестированию. При этом, на учете с диагнозом меланома состоит 2251 человек, направлено на BRAF-тестирование – 278 пациентов, мутации выявлены у 105 (37,7%).

Заключение: Внедрение молекулярно-генетического тестирования на фоне КОППА в рамках Комплексного Плана по борьбе с онкологическими заболеваниями в РК на 2018-2022 годы позволит основой для широкого и повсеместного использования молекулярно-генетических методов в Республике Казахстан. Молекулярно-генетическое тестирование при меланоме является неотъемлемым этапом диагностики и лечения злокачественных новообразований, требует обязательного проведения молекулярно-генетического исследования как при первичной постановке диагноза, так и при прогрессировании заболевания. Обнаружение мутации гена BRAF при меланоме мозг открываёт возможность терапии ингибиторами тирозинкиназ, так называемыми ингибиторами BRAF, что увеличивает вероятность ответа приблизительно на 50% и открывает новые терапевтические опции.

Ключевые слова: меланома, молекулярно-генетическое тестирование (МГТ), мутация гена BRAF V600E, персонализированный подход, молекулярно-генетическая терапия.