A Variant of c-KIT Gene in Vulvar Melanoma May Be a Chance of Molecular therapy?

Vulvar Melanomda c-KIT Gen Varyantı Moleküler Tedavi için Bir Şans Olabilir mi?

Ozbilgec S[1], Somuncu MN[2], Demir ET[1], Acar A[1]

[1]Department of Gynecologic Oncology, Necmettin Erbakan University, Meram Medical Faculty, Konya, Turkey

[2]Department of Medical Genetics, Necmettin Erbakan University, Meram Medical Faculty Konya, Turkey

Corresponding author:

Somuncu MN, Necmettin Erbakan University, Meram Medical Faculty, Medical Genetics, Turkey, Konya,

E-mail: mnsomuncu@gmail.com,

Phone: 05056403797/ 03322237311

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Abstract

Vulvar nodular melanoma is a rare type of vulvar cancer. We reported a patient with vulvar nodular melanoma who had previously been operated on breast cancer. The case was prepared to share pathological and medical genetic examinations and treatment of this case with professionals dealing with oncology. We studied somatic mutation panel on biopsy material by next gene DNA sequence technology. According to molecular genetics data, a pathogenic variant of the c-KIT gene was detected which had a therapeutic target in metastatic melanoma.
Key words: Breast cancer, c-KIT gene, vulvar melanoma

Özet

Vulvar nodüler melanom, nadir görülen bir vulvar kanserdir. Bu vakada meme kanseri öyküsü ile opere olmuş vulvar nodüler melanomlu bir hastayı sunduk. Olgu sunumu, patoloji, tıbbi genetik ve tedavisini yönlendiren jinekoljik onkoloji tarafından derlenmiştir. Hastada, yeni nesil DNA sekans teknolojisi ile somatik mutasyon paneli çalışılmıştır. Moleküler genetik analiz sonuçlarına göre, metastatik melanomda terapötik bir hedefi olan c-KIT geninin patojenik bir varyantını tespit ettik.
Anahtar sözcükler: Meme kanseri, c-KIT geni, vulvar melanom

Introduction

The most serious form of skin cancer is cutaneous melanoma. It is the fifth most common cancer in both genders in the United States and the incidence increases with age. The second most common vulvar cancer histology is melanoma, accounting for approximately 2 to 10% of primary vulvar neoplasms. The second most common type of melanoma is nodular melanoma, accounting for 15 to 30% of all melanomas (1). We present here a single case of a vulvar nodular malign melanoma patient with previous ductal breast cancer.

Mutations are the most important factor in the development and progression of carcinogenesis. Therefore, some mutations are the genetic signature in the diagnosis or the molecular marker for the therapy in cancers. Breast cancer 1 (*BRCA1*) and breast cancer 2 (*BRCA2*) have a high penetrance as tumor suppressor genes responsible for repairing DNA. When mutations or pathogenic variants are detected in *BRCA1* and *BRCA2* genes, the risk of cancer, especially breast (80%) and ovarian cancer (40%), increases. Additionally, age of diagnosis, familial predisposition, and multiple tumors are the higher risk factors for the pathogenic variants of *BRCA* genes. However, vulvar melanomas are a rare type of gynecologic cancer with a poor prognosis. Besides, based on the literature on the mutational profiles of vulvar melanoma and cutaneous melanoma, while murine sarcoma viral (v-RAF) oncogene homolog B1 (*BRAF)* mutations are the most common (50-60%) in cutaneous melanoma, the mutations of *c-KIT* and *NRAS* (27.6%) have been stated as the most common in vulvar melanoma (2).

Case

In the current report, we presented a case with vulvar melanoma and breast cancer history presenting with *c-KIT* mutation although no pathogenic variant of *BRAF, NRAS,* and *BRCA1/2* genes was detected. Therefore, we aimed to report the case for the pathological, surgical, and genetic determination of the *KIT* mutation as a therapeutic target in metastatic melanoma (3). A 59-year-old postmenopausal, gravidity 4 (G4) woman had been diagnosed with ductal breast carcinoma six years ago, and so undergoing post-modified radical mastectomy and receiving chemotherapy currently (Figure 1). The putositron emission tomography-computed tomography (PET/CT) scan showed the increased F-18 fluorodeoxyglucose (FDG) uptake in the vulvar region, and the investigation revealed a vulvar nodular lesion of 3x4 cm diameter noticed on the upper side of the left labium majus. The lesion was colorless and shiny. There was ulceration on the surface. The endometrial biopsy demonstrated that there were nondiagnostic fibrin scraps, and the cervical smear test also showed atrophy and intense signs of inflammation.

The patient underwent the local excision of the lesion. The surgically removed specimen was sent to the pathology department for the frozen section analysis. In the frozen section analysis, a malign pathology was observed. Therefore, the bilateral inguinal lymphadenectomy was performed for the case. The final pathology revealed that the patient had a nodular type of cutaneous melanoma. The maximum tumor thickness (Breslow) was measured as 0.5 cm. The presence of microsatellites was not observed in the existing sections, and the peripheral surgical margins were reported to be adjacent to the tumor. The deep surgical margin was measured to be 0.5 cm away, and the rate of mitosis was 33 per mm^2. Additionally, the anatomical stage (Clark) was defined as 5, and there was a lymph vascular invasion. Even so, no neurotropism was observed in the available sections. Tumor-infiltrating lymphocytes were prominently present; however, no tumoral regression was witnessed in the available sections. Thus, the pathological staging was noted as pT4bNxMx. Immunohistochemically, the types of tumor cells were detected as vimentin(+), melan-A(+), S-100(+), BCL-2(+), HMB-45(+), pan-cytokeratin (-), P40(-), P16(-), MDM-2(-), CD31(-), CD99(-), CD34(-), CDK-4(-), myogenin (-), EMA(-), actin(-), and desmin(-) (Figure 1). The index of KI67 was found to be approximately 60%.

The malignant melanoma metastasis was observed in one of seven lymph nodes excised from the left inguinal region. The diameter of the metastatic lymph node was 2 cm, and there was an extranodal spread. Seven lymph nodes excised from the right inguinal region were noted as reactive. Genetic studies were also performed in the case. The specialists from the medical genetics and gynecologic oncology departments were consulted for the case, and the pedigree was analyzed first through a detailed family history (Figure 2). In line with the pedigree analysis of four generations, no one was detected to have a history of breast cancer; it was also seen that there was no carcinogenesis significantly, except for the history of lymphoma in the patient's uncle. Therefore, because the patient was not <50 years of age at the diagnosis age of breast cancer, the risk of hereditary breast cancer was reduced, and the case was diagnosed with vulvar melanoma, along with the history of breast cancer; the pathological tissue sample was investigated by the new-generation sequencing (NGS) assay for the somatic cancer DNA sequencing analysis. The NGS gene kit (ONCO/Reveal Multi Cancer Panel, Pillar Biosciences Inc., Natick, MA, USA) is composed of 60 genes as *ABL1, CSF1R, FGFR1, HNF1A, KRAS, NTRK1/2/3, ROS1, AKT1, APC, BRAF, CDKN2A, CTNNB1, EGFR, ERBB4, FBXW7, FGFR2, HRAS, FLT3, IDH2, GNA11, JAK3, GNAS, c-KIT, MAP2K1, PDGFRA, MLH1, PTEN, FGFR3, SMAD4, ALK, DDR2, IDH1, MET, PIK3CA, SMARCB1, ATM, ERBB2, FOXL2, MPL, PTPN11, SRC, NOCTH1, RAC1, NRAS, RET, VHL, STK11, CDH1, EZH2, GNAQ, KDR,*

NPM1, RB1, TP53, MYC, CCNE1, SMO. Additionally, *BRCA1* and *BRCA2* genes were sequenced using the peripheral blood sample with the NGS platform of the Miniseq Sequencing System produced by Illumina. All of the genes were analyzed in the 'pillar biosciences variant analysis toolkit' current version 2020.2.2.

In terms of the findings of the NGS analysis, we reported the clinical significance of the variants, except for the benign variants. For somatic mutation detection, the recommended allele frequency was found as 1%; however, the variants <1% were not reported. Although the variants of NP_009225.1:p.Met1652Ile, NP_009225.1:p.Pro871Leu, and NP_009225.1:p.Asp693Asn (the lengths of frequency: 1,18; 92,1; and 54,48, respectively) were the missense variants for BRCA1, all of those were benign. In our study, while no pathogenic variants in BRAF and NRAS genes were analyzed, the mutation of NM_000222.2:c.2466T>G (p.Asn822Lys) was investigated in the c-KIT gene, and the frequency of the G allele instead of the wild type T allele was found 44% in the case (Figure 3). The classification of the pathogenicity of all variants analyzed was based on the guidelines under The American College of Medical Genetics and Genomics (ACMG) for solid tumors. Such databases as the Catalogue of Somatic Mutations in Cancer (COSMIC), My Cancer Genome, VarSome, Franklin, and the Clinical Interpretation of Variants in Cancer (CIViC) were used to detect the variants in somatic cancers. Also, BRCA1 and BRCA2 have been analyzed under the National Comprehensive Cancer Network (NCCN) guidelines and the germline databases such as ClinVar (4).

Discussion

In the patient we reported, the concomitance of a vulvar nodular malignant melanoma with previous ductal breast cancer and rheumatoid arthritis makes this case intriguing. Vulvar melanomas are predominantly witnessed among postmenopausal, non-Hispanic Caucasian patients at a median of 68 years of age (ranging between 10-99 years) (5). Proper surgical treatment is critical for the diagnosis, staging, and optimal management of primary cutaneous melanomas. In our case, peripheral surgical margins were determined to be adjacent to the tumor; therefore, our case underwent the re-excision procedure. The pathology report related to the re-excision procedure stated that there was no residual tumor.

The goals of the surgery include the histologic confirmation of the diagnosis, ideally carried out by an appropriately scheduled biopsy before the final surgical treatment. The wide excision of the primary melanoma obtains a complete and accurate pathologic staging of the primary tumor to guide further treatment and management. The wide excision of the primary

melanoma site with an adequate margin of normal tissue around the primary site is performed to minimize the risk of local recurrence.

Vulvar melanomas are important types of melanomas with genetic characteristics. The fact that as a rare type of cancer, the vulvar melanoma was observed in our case with the history of breast cancer first led us to consider the risk of predisposition to carcinogenesis. However, the diagnosis of breast cancer when the patient was over 50 years of age and the absence of no significant malignancies in the pedigree caused us to leave the hereditary cancer approach. Therefore, we evaluated the somatic cancer genes by NGS for the molecular etiology of cancer in our patients. Based on the literature, first BRAF, NRAS, c-KIT, and also other genes in the somatic cancer sequencing panel are known as the prognostic markers for vulvar melanomas (6). In our study, we analyzed no pathogenic variant in BRAF and NRAS genes; however, NM_000222.2:c.2466T>G (p.Asn822Lys, N822K) mutation was detected in the c-KIT gene.

c-KIT is a proto-oncogene having great importance in cell growth and proliferation. However, if a gain-of-function mutation occurs mostly in this gene, the tumorigenesis is induced by oncogenic c-KIT in vulvar melanoma. In the study by Wohlmuth and Wohlmuth Wieser (2021), the mutation profile of c-KIT was reported on exons in vulvar melanoma, and the affected coding sequence for c-KIT mutations are the incidences of 66.7% on exon 11, 19% on exon 13, 11.9% on exon 17 and 2.4% on exon 9, respectively (7). The N822K mutation analyzed in our case was on exon 17 and classified as tier 1/2, which has a strong/potential clinical significance. Thanks to the evidence for N822K, the variant is of therapeutic, prognostic, and diagnostic importance. We consider that the ‘tier 1 level B or tier 2 level C’ category is for the variant; thus, various FDA-approved therapies have been reported for different tumor types, especially for vulvar melanomas by the American College of Medical Genetics and Genomics (ACMG).

Oncogenic c-KIT has been observed in many tumor types, especially in gastrointestinal stromal tumors (GISTs), lung cancer, acute myeloid leukemia, and also melanomas. KIT variants have been observed in 6.55% of those with melanomas; however, the N822K variant has been reported in 0.26% of all melanomas. Under the classification databases of the variants, ‘Sorafenib’ has evidence in treating melanoma, and so can be the target therapeutic modality for the N822K variant. Sorafenib, designed to block tumor cell growth, is a multikinase inhibitor targeting cancer therapy, and its mechanism inhibits cell growth and proliferation and thus tumor progression in melanomas (8).

The identification of somatic mutations in those with melanoma will shed light on the identification of the genetic pathways targeting pathogenic variations as therapeutic targets. In

Turkey, the elderly neglect and are late in consulting healthcare professionals or seeking medical assistance because of the lesions in their vulvar regions. Therefore, when such patients are diagnosed, their diseases are at advanced stages. Our case also displayed such a situation; therefore, the condition was diagnosed and treated at an advanced stage.

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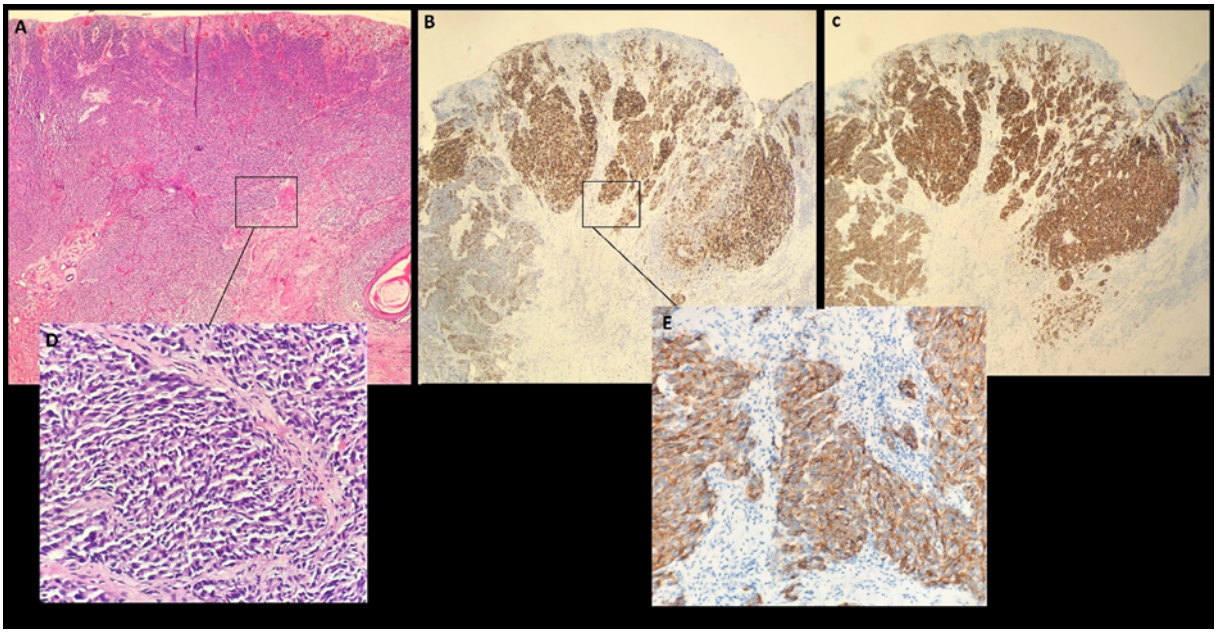

Figure 1. A) X40, H&E; Infiltration of malignant melanoma along the entire dermis is seen on the skin of the vulva, B) HMB45, X100; Tumor reacts extensively with HMB45, C) MART1, X100; Tumor shows positive reaction on MART1 immunohistochemistry staining, D) X400, H&E; Close-up view of atypical malignant melanoma cell clusters, E) HMB45, X400; The reaction of tumor cells in HMB45 immunostaining is seen closely.

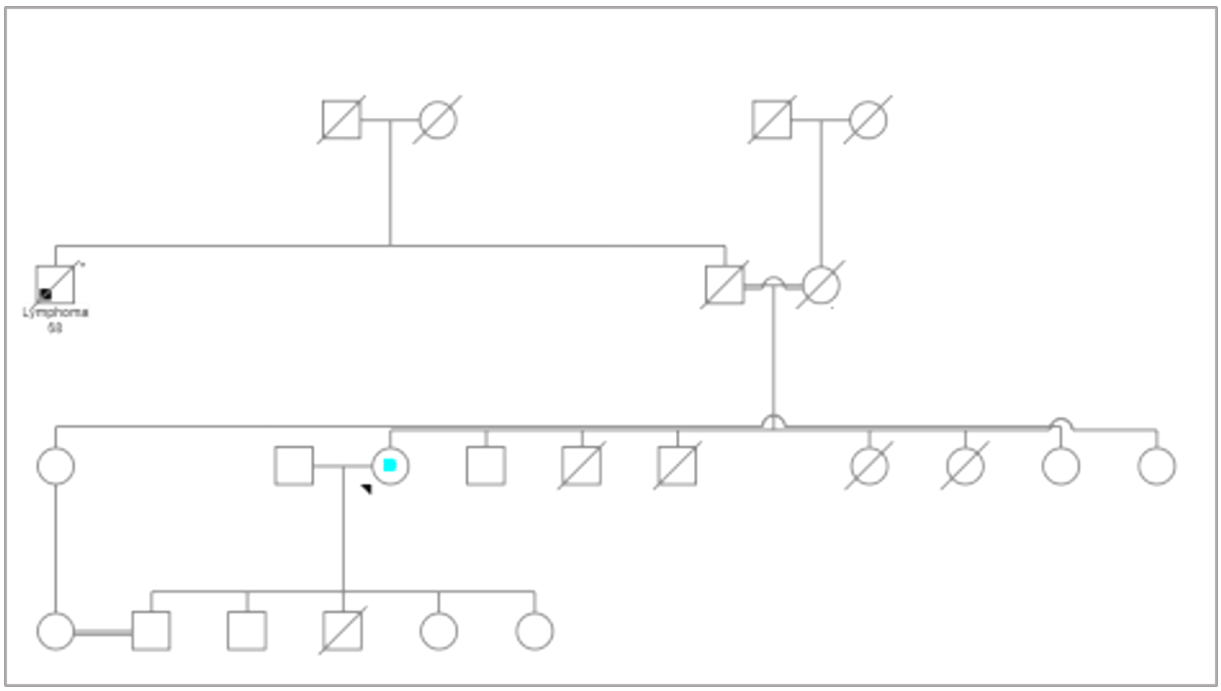

Figure 2. Pedigree chart four generations of the proband. No breast cancer or vulvar melanoma was observed in the generations.

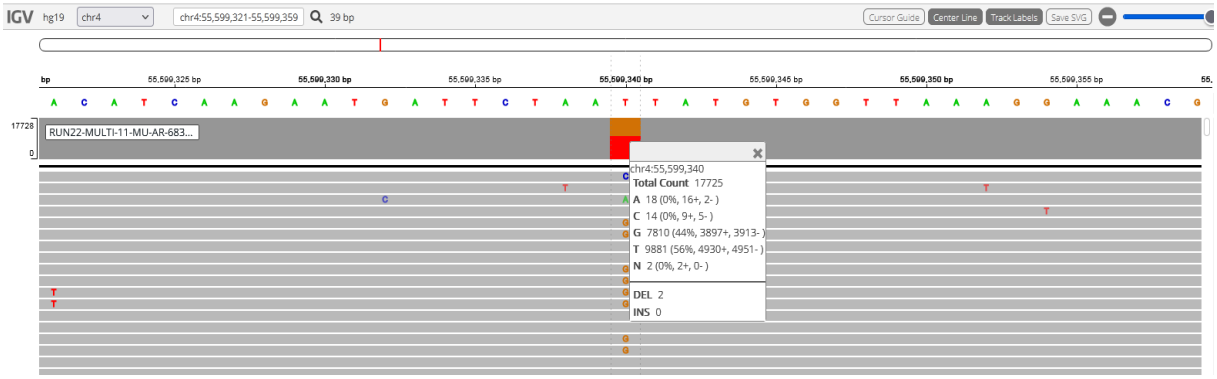

Figure 3. NGS data of KIT mutation. NM_000222.2:c.2466T>G (p.Asn822Lys) mutation was detected 44%, cut-off allele frequency for somatic variant: 1% in NGS