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Case Study

BRAF Mutation in Albanian Melanoma Patients
New Approach from Diagnosis to the Treatment

Healthcare

Keywords: Albania, melanoma, BRAF mutation, biopsy.

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Abstract

The incidence of cutaneous melanoma is steadily increasing, mainly in populations of European origin and is thus an important public health issue throughout the world. Cutaneous melanoma is a malignancy with a favorable prognosis if it presents as a localized disease, but with a dramatically worse prognosis if it metastasizes. No effective treatment exists for the group of melanoma patients with metastasizing disease, although several nonspecific chemotherapy regimens and immunotherapies are able to prolong survival at least for a short period of time. Last years has started a new area in the treatment using BRAF mutation inhibitors, especially for patients with BRAF V600E positive, so, also in melanoma is available targeted therapy. This new treatment plays an important role in their survival. *The aim of the study*: to evaluate the BRAF mutation in patients diagnosed with melanoma. *Methods*: We analised BRAF mutation in 12 formaline -fixed paraffine embedded (FFPE) tissue of patients diagnosed with melanoma from January to December 2014. *Results*: From 12 FFPE tissue we found 67 % (n=8) BRAF V600E, 25 % (n=3) BRAF Wild Type, 8% (n=1) BRAF V600K. *Conclusions*: BRAF V600E mutation is an important molecular target for novel therapeutic approaches in area of targeted therapy of melanoma patients. Patient with unresectable or metastatic melanoma, whith BRAF V600E, can use BRAF – inhibitors, increasing their survival.

Introduction

Melanoma is a malignant pathology, accounting approximately 4% of skin cancers (1). Melanoma is the most aggressive form of skin cancer, it remains highly curable if it is detected early: 5-year survival is approximately 90% overall and exceeds 98% when disease is detected at the localized stage (84% of cases) (2).

The treatment of choice for early melanoma, is no doubt surgical resection, and adjuvant therapy with alfa interferon in stage II and III of the disease (3). In advanced stage, melanoma is an aggressive disease with poor prognosis.

Last years new therapeutic approaches comes in this field using BRAF V600E inhibitors for melanoma patients.

BRAF is a serine threonine kinase and a member of the MAPK – pathway (Mitogen – activated protein kinase), which is responsible for cell growth, survival, and differentiation. BRAF is highly expressed in melanocytes and neuronal tissue, both of which are of neural crest origin.

BRAF gene mutation testing has an important tool for diagnosis, prognosis, treatment, and predicting patient outcome in response to targeted therapy for multiple cancer types (4). Since August 2011, FDA (Food Drug administration) has approved Vemurafenib (Zelboraf) – an oral inhibitor of BRAF

mutation, for patients with metastatic melanoma or unresectable melanoma who have BRAF V600E mutation (5).

Which patients should be tested?

Option 1: Test all melanoma patient should be tested for BRAF mutation, regardless of stage, because BRAF mutation status does not change between primary or metastatic melanoma (6,7).

Option 2: Test Patients (Stage IIb or IIc) who have a risk of recurrence 40–70% , mainly 2–4 years after the initial diagnosis (8).

Option 3: Test Patients diagnosed with Metastatic melanoma.

Methods

In this study were involved 12 patients diagnosed during 2014, with cutaneous malignant melanoma, or lung metastatic melanoma in the department of pathology in the University Hospital Center "Mother Teresa" and in the University hospital "Shefqet Ndroqi" – Tirana. Method used for evaluation of BRAF mutation, in these FFPE tissue was Sanger Sequencing in the department of molecular biology and pathology (University of Ferrara).

Sanger Sequencing: PCR amplicons were generated usinë the following primers: 50 AGGTGATTTTGGTCTAGCTACAG–30 and reverse: 50 GTTGAGACCTTCAATGACTTTCTAG–30, and were analyzed on the ABI Genetic Analyzer 3130 XL (Applied Biosystems, Foster City, CA), using the Big–Dye terminator kit v1.1 (Applied Biosystems).

Analysis is made using SPSS, version 16.0. A p value less than 0.05 was considered to be statistically significant.

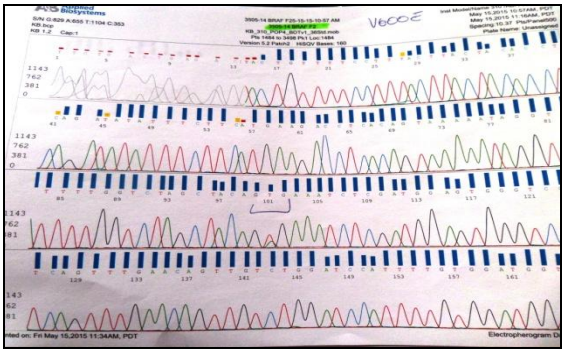
Results

This study included 12 albanian melanoma patients. Patients were analised according to male female ratio, Breslow thickeness, Clark level, angiovascular invasion, ulceration and BRAF status. The table belows shows all clinicopathologic findings (table 1):

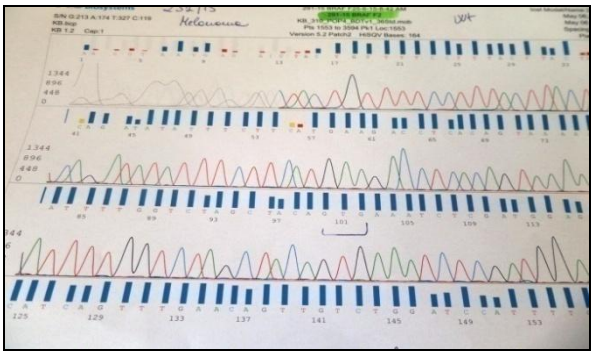
Table 1 Clinocopathological Characteristics of the 12 Study Patients	
Characteristic	No. (%)
Male	8 / 67%
Female	4 / 33%
Mean age	66.3 years
Range (min-max)	Min=45 / Max=90
Clark level mean	3
Breslow mean (mm)	3,2
Ulceration	
Yes	7 / 77%
No	2 / 23%
Histological type	
Nodular melanoma	6
Superficial spreading	3
Metastasis	3
Angiovascular invasion	

Yes	7 / 59%
No	5 / 41%
Resection margins	
Yes	10 / 84%
No	2 / 16%

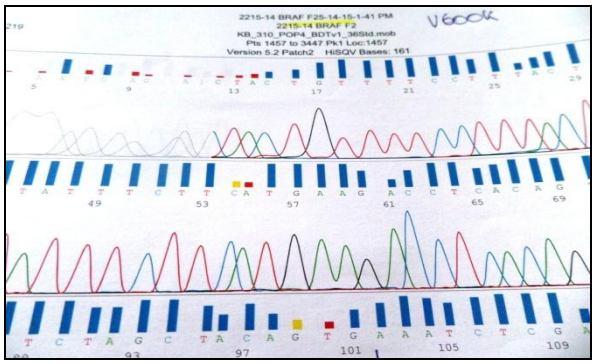
12 FFEP tissue underwent Sanger Senquencing and we observed that 67 % (n=8) of patients were BRAF (V600E), 25 % (n=3) were BRAF WT (wild type), 8 % (n=1) were BRAF (V600K), illustrated as in figures below (Figure 1):



a



b



c

Figure 1 - a – V 600E codon resulting from a single – nucleotide mutation (GTG > GAG).
- b – V600 codon showing the wild – type GTG sequence.
- c – V600K codon resulting from a dinucleotide mutation (GTG > AAG).

Table 2

Age	sex	Diagnoza	type	BRAF/V600E	BRAF/V600K	BRAF/WT
80	f	melanoma	nodulare	positive		
67	m	metastasis				Positive
73	f	melanoma	nodulare		positive	
67	m	melanoma	superficial spreading	positive		
53	m	melanoma	nodulare	positive		
45	m	melanoma	nodulare	positive		

73	f	metastasis		positive		
49	m	melanoma	nodulare			Positive
72	f	melanoma	superficial spreading			Positive
68	m	melanoma	superficial spreading	positive		
90	m	melanoma	nodulare	positive		
40	m	metastasis		positive		

Considering histological type or metastatic melanoma, the BRAF V600E mutation was detected in 4 (33%) cases of nodular melanoma, 2 (17%%) cases of superficial spreading melanoma and 2 (17%) cases in metastasis of lung (table 2).

Discussion

The aim of our study was to evaluate BRAF mutation in Albanian melanoma patients and also, to investigate possible associations between prognostic parameters such as Breslow depth, Clark level, age, gender, ulceration, and the presence of BRAF mutation, even the number of samples is limited, because of the cost of the examination.

There is a significative correlation between Breslow and Clark level ($p<0.05$), but we did not find any statistically significant association between the presence of the mutation and histological subtype, gender, or age.

We found statistically significant association between ulceration and angiovascular invasion ($p= 0.034$). There is no significant association between BRAF mutation and the presence of ulceration, or angiovascular invasion.

In our study the most common type was nodular melanoma while in literature superficial spreading melanoma was the most common histological subtype (9,10). In our study BRAF V600 E was found in 67% of cases. The table below (Table 3) shows a review of literature about BRAF V600 E.

Literature review of different studies that investigated BRAF mutations in melanomas.		
Reference	**No. of subjects/country**	**BRAF V600E mutation frequency (%)**
Davies et al., 2002 (11)	34/USA, Italy, Hong Kong, England	55.9
Gorden et al.2003 (12)	77/USA	40
Libra et al.2005 (13)	19/Italy	63
Goel et al.2006 (14)	58/USA	57
Lee et al., 2006 (15)	35/USA	60
Liu et al., 2007 (16)	251/Australia	45
Venesio et al., 2008 (17)	18/Italy	72
Viros et al., 2008 (18))	302/USA,Germany,Japan,South Korea	47
Casula et al., 2009 (19)	35/Italy	31.4
Lázár et al., 2009 (20)	74/Hungary	27
Narita et al., 2009 (21)	71/USA and Australia	39
Broekaert et al., 2010 (22)	350/Germany, Austria, USA	49.7

Ellison et al., 2010 (23)	163/Great Britain	41.1
Scherer et al., 2010 (24)	179/Italy and Germany	17.3
Rubinstein et al., 2010 (25)	138/USA	69
Ellerhorst et al., 2011 (26)	223/USA	42.2
Janku et al., 2011 (27)	52/USA	50
Long et al., 2011 (28)	197/Australia	35.5
Shibata et al., 2011 (29)	39/Japan	28.2
Menzies et al., 2011 (30)	312/Australia	33.6
Inumaru at al., 2014 (9)	77/Brasil	70.1

Conclusions

The detection of BRAF mutation in melanoma, has emerged as a central factor in the stratification of melanoma patients to deploy various targeted therapies in advanced–stage disease., patients with BRAF V600E, can use BRAF–inhibitors, improving their quality of life and their survival.

Albeit the sample is relatively small and the time length of the study is short, it is worth mentioning that other financial support are needed to improve our research in the area of molecular events of Albanian melanoma patients, to help and improve their quality of life.

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