Experimental and Molecular Pathology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Comparing BRAF mutation status in matched primary and metastatic cutaneous melanomas: Implications on optimized targeted therapy

Maya Saroufim [a], Robert H. Habib [a], Rita Gerges [a], Jad Saab [b], Asif Loya [c], Samir S. Amr [d], Salwa Sheikh [e], Mohammad Satti [f], Christian Oberkanins [b,g], Ibrahim Khalifeh [a,*]

[a] American University of Beirut Medical Center, Beirut, Lebanon
[b] Weill-Cornell Medical Center, New York, NY, United States
[c] Shaukat Khanum Memorial Cancer Center and Research Hospital, Lahore, Pakistan
[d] King Fahad Specialist Hospital, Dammam, Saudi Arabia
[e] Dhahran Health Center, SAMSO, Dhahran, Saudi Arabia
[f] King Abdul-Aziz Medical City, Jeddah, Saudi Arabia
[g] ViennaLab Diagnostics GmbH, Vienna, Austria

ARTICLE INFO

Article history:
Received 20 August 2014
Accepted 12 September 2014
Available online xxxx

Keywords:
BRAF
Matched primary and metastatic melanomas
Targeted therapy

ABSTRACT

Background: Selective BRAF inhibitors have shown dramatic results with regard to improving outcome in patients with melanoma. Testing the BRAF status in matched primary and metastatic melanomas to optimize individual targeted therapy is not well investigated.
Methods: Extended BRAF testing using PCR for 9 mutations and VE1 immunohistochemistry for BRAF V600E detection on 95 lesions including 40 primary melanomas with their matched metastases ($n = 42$), recurrences ($n = 9$) and second primaries ($n = 4$) was performed. Nine patients had multiple metastases.
Results: V600E was the only identified mutation type; 35.4% of primary vs. 18.9% of metastatic melanomas. The overall primary-metastatic BRAF status discordance rate was 32.3% using PCR and 27.5% with immunohistochemistry, and was significantly more frequent in primary lesions with mutant BRAF (67%). Males and patients with metastasis to lymph nodes were less likely to be discordant compared to females and those with metastasis to other sites ($p = 0.023$). Discordant BRAF mutation status was predicted by multivariate binary logistic regression: the presence of a mutant BRAF in the primary melanoma [OR (95% C.I.) = 23.4 (2.4–229.7)] and female gender [OR = 10.6 (1.08–95)]. Inter-metastases BRAF concordance was 100% (6 comparisons).
Conclusion: A high discordant rate implies the need for clinical trials addressing the response to targeted therapy in patients with discordant BRAF statuses between their primary and metastatic lesions.
© 2014 Published by Elsevier Inc.

1. Introduction

The mortality and morbidity associated with melanoma continue to increase at a staggering rate, with reports of a 7% yearly increase in risk of death from melanoma seen from 1990 to 2006 (Jemal et al., 2011). The observation of high mutation rates in the BRAF oncogene in melanoma and melanocytic nevi (Karram et al., 2012; Pollock et al., 2003; Saroufim et al., 2014a, 2014b) has shed new light on the understanding of melanoma biology. As our knowledge continues to increase and the behavior of these tumors becomes clearer, the current advances in the molecular profiling of melanoma give hope in providing novel, and possibly more effective treatment options in the management of this deadly disease.

BRAF mutation, as an early and fundamental feature of melanocytic neoplasia, through the mutational activation of the RAS/RAF/MEK/ERK pathway and subsequent mediation of cellular responses to growth signals (Davies et al., 2002; Peyssonnaux and Eychene, 2001), has been suggested to be a critical step in the development of melanocytic tumors. The majority of mutations in the BRAF gene affect codons 600 and 601, with V600E (c.1799 T > A) being by far the most frequent aberration. The BRAF V600E mutation is one of the most common kinase domain mutations in human cancer with a particularly high incidence in malignant melanoma (Curtin et al., 2005; Davies et al., 2002; Fecher et al., 2008; Wan et al., 2004). In melanoma cells, oncogenic BRAF has been found to play a key role in the regulation of pro-apoptotic proteins (Boisvert-Adamo and Aplin, 2008; Cartlidge et al., 2008; Shao and Aplin, 2010), and in driving the invasion of melanoma cells through increased expression of the pro-migratory beta-3 integrin receptor, upregulation of matrix metalloproteinases and the release of cytosolic calcium (Arozarena et al., 2011; Smalley, 2003).

* Corresponding author at: American University of Beirut Medical Center, Department of Pathology & Laboratory Medicine, Cairo Street, Hamra, Beirut 1107 2020, Lebanon.
E-mail address: ik08@aub.edu.lb (I. Khalifeh).

http://dx.doi.org/10.1016/j.yexmp.2014.09.008
0014-4800/© 2014 Published by Elsevier Inc.

Please cite this article as: Saroufim, M., et al., Comparing BRAF mutation status in matched primary and metastatic cutaneous melanomas: Implications on optimized targeted therapy, Exp. Mol. Pathol. (2014), http://dx.doi.org/10.1016/j.yexmp.2014.09.008

ARTICLE IN PRESS

Given that greater than 90% of BRAF mutations in melanoma are of the V600E type (Davies et al., 2002), recent clinical trials have shown dramatic results with the use of Vemurafenib, a selective BRAF V600E inhibitor, in improving overall and progression-free survival in patients whose tumors harbor the BRAF V600E mutation (Chapman et al., 2011). Similarly, in the pivotal phase III trial, 250 patients with unresectable stage III or stage IV melanoma were randomly assigned to either Dabrafenib, another selective BRAF inhibitor (150 mg orally twice a day) or Dacarbazine (1000 mg/m^2 IV every three weeks) with Dabrafenib showing significant increase in progression-free survival (Hauschild et al., 2012). Dabrafenib was approved by the US Food and Drug Administration in May 2013 for the treatment of patients with advanced melanoma harboring the BRAF V600E mutation. LGX818 is another potential therapeutic agent in its phase III clinical trials, disclosed at the spring 2013 American Chemical Society meeting in New Orleans, and also appears promising in treating these patients.

The use of targeted therapy is based on the assumption that metastatic tumors are primarily clonal with respect to the mutant oncogene. However, the presence of different genetic alterations in different metastatic tumors from the same patient has been reported in lung and esophageal cancer as well as melanoma (Katona et al., 2007; Maley et al., 2006; Taniguchi et al., 2008). Such genetic heterogeneity is important in the advent of targeted therapy in predicting drug efficacy and patient response.

The analysis of the molecular profile of matched primary and metastatic melanomas in the same patient is fairly new. Reports on the presence of different genetic alterations in metastatic tumors from a single patient, i.e. "inter-tumor heterogeneity", warrant investigations of matched primary and metastatic melanomas to determine whether BRAF mutation status is concordant or discordant in the same patient. Rates of discordance, if significant, would then mandate genetic profiling of patients with both primary and metastatic lesions before the selection of an appropriate treatment modality. Only a handful of studies are currently available on this topic (Omholt et al., 2003; Shinozaki et al., 2004; Yancovitz et al., 2012) and more data is needed to solidify the findings in the literature, especially when it comes to the implications of these findings on selecting patients for testing and new therapeutic modalities. Our study examines a series of patients with matched primary and metastatic melanomas, second primaries and recurrences. In addition to previous studies, we sought to identify potential factors that may affect concordance rates in these patients. Furthermore, we addressed the issue of inter-metastasis concordance. Do patients with multiple metastases have the same genetic profile in all their metastatic lesions? If they do, or do not, what are the implications? And how do we interpret all of our results in the context of emergent targeted therapy for melanoma?

2. Materials and methods

2.1. Sample selection and clinical data

This study was approved by the American University of Beirut Medical Center institutional review board (IRB: PALM.IK.02). A total of ninety five formalin-fixed paraffin-embedded (FFPE) tissue blocks of matched cutaneous melanoma including primary and metastatic lesions and recurrences, microscopically diagnosed between 1996 and 2012, were collected from the Pathology and Dermatology archives at the American University of Beirut, Lebanon, Shaukat Khanum Memorial Cancer Center and Research Hospital, Lahore, Pakistan, Dhahran Health Center, Saudi Arabia and King Abdul-Aziz Medical City, Jeddah, Saudi Arabia. All slides were reviewed and marked for microdissection. Locations of the primary lesions were divided into the upper extremity, lower extremity, head and neck, trunk (including inguinal lesions) and "others". The number and location of metastasis for each primary were recorded and divided into metastasis to lymph nodes, subcutaneous tissue or "others". All metastases to lymph nodes were clinically palpable lymph nodes. The presence of a second primary and/or recurrence was also documented. Clinical parameters including gender, age and the period of time between excision of the primary lesion and the development of a metastasis were recorded. Cases with incomplete clinical data, insufficient material for PCR and patients previously treated with BRAF targeted therapy were excluded from the study.

2.2. DNA extraction and extended BRAF mutational testing

DNA was extracted from FFPE tissue using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). BRAF genotyping was performed by PCR followed by reverse hybridization as described for teststrip assays. Briefly, a DNA fragment spanning BRAF codons 600–601 was amplified and biotinylated by PCR using primers described elsewhere (V. Buxhofer-Ausch et. al., manuscript submitted). Amplicons were hybridized for 30 min at 45 ± 0.5 °C to a membrane teststrip presenting a parallel array of allele-specific oligonucleotides for each of the nine BRAF mutations: V600A (c.1799 T > C), V600D (c.1799_1800TG > AT), V600E (c.1799 T > A), V600E (c.1799_1800TG > AA), V600G (c.1799 T > G), V600K (c.1798_1799GT > AA), V600M (c.1798G > A), V600R (c.1798_1799GT > AG) and K601E (c.1801A > G). After a series of stringent washes, specifically bound PCR fragments were detected using a streptavidin–alkaline phosphatase conjugate and color substrates (BCIP/NBT). The entire hybridization and detection procedure was carried out fully automated using a temperature-controlled teststrip processor (ProfiBlot II T 48; Tecan, Groedig, Austria). Reference DNA samples previously typed by direct DNA sequencing were available for all SNPs and were used for performance control of the assay using serial dilutions of BRAF-mutated cell lines, the assay was shown to detect 1% mutated in a background of wild-type DNA.

2.3. VE1 immunohistochemistry for BRAF V600E detection

FFPE melanoma cases used for immunohistochemistry (IHC) included the blocks used for BRAF mutational testing in all patients. Four micrometer sections for hematoxylin and eosin (H&E) and VE1 staining were cut. IHC was performed on an automated system (Ventana BenchMark ULTRA; Ventana Medical Systems Inc., Tucson, AZ) utilizing the DAB IHC Detection Kit.

All H&E and IHC stained slides were evaluated in a consensus conference by tow pathologists (M.S. and I.K.), blinded to the clinical and mutational data. H&E were reviewed to confirm the histologic presence of melanoma and cytoplasmic staining of BRAF V600E VE1 antibody staining in melanoma cells was scored as positive or negative. Weak staining of interspersed cells, monocytes, and nuclear staining was deemed negative. Early necrotic cells shown to affect the VE1 staining were excluded (Capper et al., 2011; Long et al., 2012).

2.4. Statistical analysis

Continuous variables were analyzed by t-test or Mann Whitney rank sum test as appropriate. Categorical variables were analyzed using chi-square test. A 2-tailed $p < 0.05$ was required for statistical significance. Independent predictors of BRAF positive status were derived by multivariate binary logistic regression (backward selection). A $p < 0.2$ univariate significance level was used for inclusion and factors were retained if significant at $p < 0.05$. Analyses were performed using SPSS version 19 (IBM Inc., Somers, NY).

3. Results

3.1. Patient and tumor characteristics

Pooling the resources of the 4 previously mentioned medical centers; matched primary and metastatic melanomas were collected from a total of 40 patients. The patient sample included 27 men and 13

Please cite this article as: Saroufim, M., et al., Comparing BRAF mutation status in matched primary and metastatic cutaneous melanomas: Implications on optimized targeted therapy, Exp. Mol. Pathol. (2014), http://dx.doi.org/10.1016/j.yexmp.2014.09.008

ARTICLE IN PRESS

Table 1
Patient characteristics and anatomic location/BRAF status of matched primary and first metastasis.

Patient	Age (years)	Sex	PM tumor site	BRAF status of PM	Tumor site & BRAF status of 2nd PM	1st MM site, TTM (months)	BRAF status of MM
1	73	F	Leg	WT	–	Other, 144	V600E
2	48	F	Arm	V600E	–	LN, 6	PCR failed
3	61	M	Leg	WT	–	LN, 14	PCR failed
4	40	F	Leg	WT	–	SubQ, 7	WT
5	52	F	Leg	V600E	–	SubQ, 0	WT
6	48	M	Leg	PCR failed	–	LN, 0	WT
7	57	F	Leg	PCR failed	–	LN, 0	PCR failed
8	59	F	Leg	PCR failed	–	SubQ, 9	PCR failed
9	42	M	Leg	WT	–	SubQ, 27	V600E
10	49	M	Leg	V600E	Leg, V600E	LN, 0	V600E/Neg
11	82	M	Leg	V600E	–	LN, 0	V600E
12	46	M	Arm	V600E	–	LN, 0	V600E/Neg
13	61	M	Leg	V600E	–	LN, 0	V600E
14	61	F	Leg	WT	–	LN, 5	WT
15	35	F	Trunk	WT	–	LN, 36	WT
16	51	M	Trunk	WT	–	LN, 0	WT
17	44	F	Leg	WT	–	SubQ, 15	WT
18	75	M	Trunk	WT	–	LN, 49	WT
19	56	M	Head &Neck	WT	–	LN, 0	WT
20	75	M	Leg	WT	–	LN, 0	WT
21	59	M	Trunk	WT	–	Other, 11	WT
22	57	M	Trunk	WT	–	Other, 2	WT
23	72	M	Trunk	WT	–	LN, 48	WT
24	59	M	Trunk	WT	–	LN, 35	WT
25	65	M	Leg	WT	–	SubQ, 24	WT
26	58	F	Leg	WT	–	LN, 0	WT
27	65	M	Leg	WT	–	LN, 0	WT
28	40	M	Arm	WT	–	LN, 0	WT
29	78	M	Leg	WT	–	LN, 0	WT
30	62	M	Arm	V600E	–	SubQ, 0	WT
31	72	F	Arm	V600E	–	Other, 24	WT
32	86	M	Leg	V600E	–	LN, 1	WT
33	73	M	Trunk	V600E/Neg	–	LN, 0	WT
34	30	F	Leg	WT	Leg, WT	N/A	N/A
35	66	M	Head & Neck	WT	–	N/A	N/A
36	63	M	Leg	WT	–	N/A	N/A
37	60	M	Leg	V600E	Arm, WT	N/A	N/A
38	53	M	Trunk	V600E	Trunk, V600E	N/A	N/A
39	58	M	Head & Neck	V600E	–	N/A	N/A
40	67	F	Trunk	PCR failed	–	N/A	N/A

LN: lymph node; SubQ: subcutaneous; PM: primary melanoma; MM: metastatic melanoma; TTM: time to metastasis; F: female; M: male; WT: wild type; time to metastasis = 0 implies patient presented with metastasis at time of first excision; N/A: not available (patients 35, 36, 39 and 40 only had recurrent lesions available for testing, see Table 2; patients 34, 37 and 38 only had multiple primary sites available for testing). Neg: reflects the patients that were negative for BRAF using IHC, but positive for BRAF V600E by PCR. All remaining cases gave identical results using both PCR and IHC. All patients that failed PCR were found to be concordant using IHC.

UNCORRECTED PROOF

women with a mean age of 59 ± 13 years. The incidence of mutant primary lesions was less in females than in males (2/9, 22% vs. 10/25, 40%). Our cases included 40 primary melanomas with their matched metastatic lesions (n = 42). Primary tumors, including second primaries (n = 4), were located predominately on the lower extremities (24/44, 54.5%). The most common location for a first metastasis was a lymph node (22/40, 55%), followed by the subcutaneous tissue (8/40, 20%) [Table 1]. Nine cases had documented lesion recurrences, and their time to recurrence reached up to 36 months with an average time of 13 months [Table 2]. Multiple metastatic sites were present in 6 patients (4 patients had 2 sites, 1 patients had 3 sites and 1 patient had 4 sites). Patient # 2, with 4 metastatic lesions, was metastasized consistently to the subcutaneous tissue while patient # 6, for instance, was primarily metastasized to the lymph node and had subsequent subcutaneous metastasis. The time to metastasis reached up to 169 months with an average time of 13.8 months [Tables 1 and 3].

3.2. BRAF mutation status in matched primary and metastatic lesions (Fig. 1)

BRAF mutation status was obtained for 85/95 (89.5%) lesions. The PCR failure rate was 10.5% (10/95). This resulted in a total of 34 patients with primary melanomas and matched metastatic melanomas available for analysis, among them 21 males and 13 females. We performed an extended BRAF mutation analysis for 9 different mutations and V600E was the only identified mutation type documented in 36% (13/36) of primary melanomas and 14% (5/36) of metastatic melanomas. A significant number of matched cases (11/34, 32.3%) were discordant with respect to their BRAF status. Of note, discordant cases included among them patients with BRAF wild type primary lesions (patient 1 and patient 9 both developed BRAF V600E positive metastatic lesions [Tables 1 and 3]).

Table 2
Time to recurrence & BRAF status of recurrent lesions.

Patient	Time to recurrence (months)	BRAF status of recurrent lesion	BRAF status of primary melanoma
2	6	WT	V600E
3	14	PCR failed	WT
14	5	WT	WT
15	36	WT	WT
16	0	WT	WT
35	5	WT	WT
36	25	WT	WT
39	1	V600E	V600E
40	24	WT	PCR failed

WT: wild type.

Please cite this article as: Saroufim, M., et al., Comparing BRAF mutation status in matched primary and metastatic cutaneous melanomas: Implications on optimized targeted therapy, Exp. Mol. Pathol. (2014), http://dx.doi.org/10.1016/j.yexmp.2014.09.008

Table 3
Characteristics and BRAF status of patients with multiple metastatic lesions.

Patient	BRAF of Met-1	Met-2 location	BRAF status, TTM[a]	Met-3 location	BRAF status, TTM[a]	Met-4 location	BRAF status, TTM[a]
1	V600E	Other	V600E, 169	–	–		
2	PCR failed	SubQ	WT, 5	SubQ	PCR failed, 8	SubQ	WT, 15
3	PCR failed	LN	WT, 20	–	–		
4	WT	SubQ	WT, 13	–	–		
5	WT	SubQ	WT, 0	–	–		
6	WT	SubQ	WT, 76	SubQ	WT, 51		

Met: metastasis, SubQ: subcutaneous, LN: lymph node; WT: wild type.
[a] TTM: time to metastasis (months).

3.3. Inter-metastases concordance rate (Table 3)

Originally, 9 patients were documented with multiple metastases. Of these, 3 failed PCR. The remaining 6 patients with matched metastatic melanomas at different sites displayed a 100% concordance rate in the BRAF mutation status.

3.4. Comparison of BRAF mutation testing and VE1 IHC for BRAF V600E detection

We sought to strengthen our findings by confirming our results using VE1 immunohistochemistry for BRAF V600E detection on all of our cases. We found 3 cases to be negative using IHC, two were from the lymph nodes of patients 10 and 12 as outlined in Table 1, and one was the primary lesion of patient 33. All 3 cases, however, were positive for the BRAF mutation using PCR. We found no false-negative cases with the addition of the immunohistochemical stain. The six patients that failed PCR due to an inadequate DNA sample were found to be concordant using IHC. Therefore, the overall primary-metastatic BRAF status discordance rate was 32.3% (11/34) using PCR and 27.5% (11/40) with immunohistochemistry, still a significant discordance rate.

3.5. Determinants of concordance and discordance in matched cases

We found discordance in BRAF status both in patients with mutant BRAF primary melanomas as well as wild-type BRAF primary lesions. However, discordance was significantly more frequent in primary melanomas with mutant BRAF (8/12, 67%) versus those with wild-type BRAF lesions (3/22, 14%, $p = 0.005$). With regard to the anatomic location of the metastatic lesions, we found that patients with lymph node metastasis were less likely to be discordant compared to those with metastasis to other sites [3/20 (15%) vs. 8/14 (57.1%), $p = 0.023$]. Females had marginally higher discordant rates than males [7/13 (53.8%) vs. 4/21 (19%), $p = 0.06$). Despite our extensive analysis of time-to-metastasis, including subdividing patients into 2 groups (patients that presented with metastasis at the time of the first excision and patients that presented with metastasis at a later time) and running time as a continuous variable, no univariate associations with concordance were identified ($p = 0.55$). Similarly, in our analysis of patient age, no statistically significant association with concordance was noted ($p = 0.41$). Anatomic location ($p = 0.23$) was also not associated with rates of concordance.

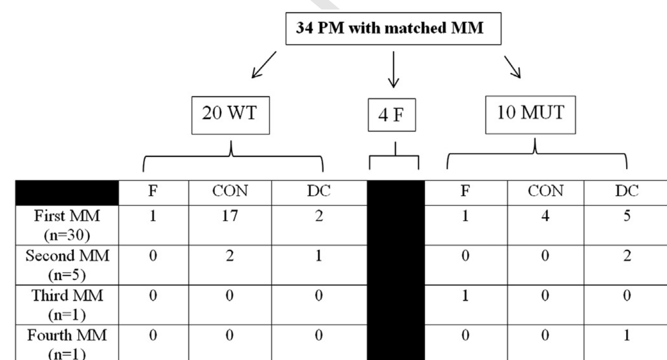

Fig. 1. Algorithm summarizing the breakdown of 34 patients' BRAF status in matched primary and metastatic melanomas. PM: primary melanoma; MM: metastatic melanoma; F: PCR failed; WT: wild type; MUT: mutant type; CON: concordant PM–MM BRAF status; DC: discordant PM–MM BRAF status.

3.6. Multivariate analysis

Multivariate binary logistic regression showed that discordant BRAF mutation status for the primary versus metastatic lesions was predicted by two factors: the presence of a mutant BRAF in PM [OR (95% C.I.) = 23.4 (2.4–229.7)] and female gender [OR = 10.6 (1.08–95)].

4. Discussion

As we delve further into the realm of personalized medicine, the role of the pathologist has evolved beyond pure histopathologic diagnosis to include analysis for the presence or absence of gene mutations, such as BRAF, so they may be specifically targeted by a therapeutic agent (Amaria et al., 2012). Improved survival in melanoma patients with BRAF V600E mutations treated with Vemurafenib and Dabrafenib shows promising results in the treatment of this devastating disease (Chapman et al., 2011; Hauschild et al., 2012). This makes it very desirable to understand the exact pattern of expression of this oncogene not only in the primary lesion but also in matched metastatic lesions in the same patient as well. The question then also arises whether multiple metastatic lesions in the same patient would harbor the same BRAF mutation status.

Studies examining whether BRAF mutation status is conserved with subsequent metastasis have yielded conflicting results. Omholt et al. (2003), in a series of 51 patients with matched primary and metastatic tumors, found that 96% of their patients expressed the same *BRAF* genotype in their primary lesion as in their corresponding metastatic lesion(s). Furthermore, if the primary tumor was wild type for *BRAF*, no mutations arose by the metastatic stage. An exception was two patients whose primary tumors were wild type for *BRAF* but corresponding metastasis contained the mutation (Omholt et al., 2003). They argue that BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. Similar high rates of concordance were observed by Menzies et al. (2010) who reported a 100% concordance rate on a series of 64 patients (Menzies et al., 2013). Shinozaki et al., however, reported a discordant mutation status in 5 out of 13 pairs (38%) of matched primary and metastatic melanomas, a much higher discordance rate than Omholt et al. (2003) and Menzies et al. (2010), and all their discordant cases had initial BRAF wild type primaries (Shinozaki et al., 2004). On the same note, Yancovitz et al. (2012), in series of 18 patients, reported a 44% discordant rate. Six of their 8 patients had wild-type primary tumors, but mutant metastatic specimens, a pattern consistent with the acquisition of the BRAF V600E mutation conferring a survival advantage for metastasis. Eleven percent of their patients had initial BRAF V600E mutant primaries but BRAF-WT metastasis (Yancovitz et al., 2012).

Please cite this article as: Saroufim, M., et al., Comparing BRAF mutation status in matched primary and metastatic cutaneous melanomas: Implications on optimized targeted therapy, Exp. Mol. Pathol. (2014), http://dx.doi.org/10.1016/j.yexmp.2014.09.008

ARTICLE IN PRESS

The first aim of our study was to compare the mutational status in primary and matched metastatic melanomas. Our results from 40 patients displayed a significant discordance rate of 32.3% (11/34) using PCR and 27.5% (11/40) by IHC, a figure close to the one previously reported by Shinozaki et al., with discordance found in patients that had both BRAF wild type or mutant BRAF primaries alike. In a similar study by Colombino et al. (2012), of the paired samples, patients with lymph node or visceral metastases displayed high rates (93–96%) of consistency between their primary and matched metastatic lesions however, significantly less consistent patterns were observed in patients with brain (80%) and skin metastases (75%) with the authors suggesting that independent subclones are generated in some patients of these patients. Furthermore, *p16CDKN2A* mutations were identified in 7% and 14% of primary melanomas and metastases, with a low consistency (31%) between secondary and primary tumor samples (Colombino et al., 2012). The variability in our results suggests several models of mutagenesis. The fact that patients who had the BRAF mutation in the primary lesion and then subsequently lost it in the corresponding metastasis support the notion that the mutation event has been acquired in primary melanoma and lost later in the metastasis. On the other hand, patients with BRAF wild-type primary tumors and BRAF V600E mutated metastatic lesions support the notion that mutations in the BRAF gene may be a late event acquired with the development of metastasis and may not necessarily be acquired early during melanoma pathogenesis, as previously thought. Furthermore, having a BRAF wild-type primary lesion and concomitant mutant metastasis supports the notion that mutations in the BRAF gene are not necessary in the cascade of increasing tumor aggressiveness, invasiveness and subsequent metastasis. Using sequence analysis of DNA extracted from different microdissected regions from the same tumor and by comparing multiple metastatic samples in the same tumor, intra-tumor heterogeneity of BRAF mutation status has been demonstrated (Yancovitz et al., 2012). Similar intra-tumor heterogeneity of BRAF expression has also been demonstrated immunohistochemically (Busam et al., 2012), therefore, this mutational heterogeneity in subclones may contribute as a possible explanation for our results. Our cohort resulted in 35.4% of primary versus 18.9% of metastatic melanomas displaying the mutation. This result is expected because we are dealing with a very selective and therefore biased sample of patients of only matched primary and metastatic melanomas and therefore the overall BRAF rates do not reflect the true population. In our previous work on the BRAF analysis of 600 different melanocytic neoplasms including 172 primary and 90 metastatic melanomas, we found higher mutation rates in the metastatic lesions, and the result from that unbiased sample is therefore probably more reflective of the true mutation rates in the population (Saroufim et al., 2014a, 2014b). The discrepancy among studies can only be explained by the particular sample population each group is studying. The bottom line is that there really is not enough data available yet on this subject in order to reach a consensus for mutation testing before the initiation of BRAF targeted therapy. Therefore, every cohort currently available is significant and valuable in the discussion of this issue in order to reach guidelines in the future that would benefit our patients.

When evaluating the currently available data, the question of what technique, IHC or PCR, is more accurate enters into the discussion. On the more technical side, the BRAF StripAssay has a proven sensitivity of 1% therefore, is IHC indeed considered more sensitive, i.e. would one detect less than 1% mutated cells by IHC staining? Long et al. seems to prove that IHC using VE1 compares well with molecular techniques, but these samples were macrodissected and high-resolution melt was used. The authors indicate that PCR is more sensitive than IHC, where out of 37 PCR BRAF positive cases, 2 cases were missed by immunohistochemistry staining for VE1 antibody and clinical use of the V600E BRAF antibody should be a valuable supplement to conventional mutation testing (Long et al., 2012). Having said that, we should emphasize the fact that a major aim of our study was not only to test for V600E, but also for 8 other BRAF mutations, that VE1 do not detect and that the use of both techniques together provides a possible more valuable result.

The second issue we tackled was that of identifying possible factors that may predict concordance or discordance alike. Indeed, we were able to find that discordance was significantly more frequent in primary melanomas with mutant BRAF versus those with wild-type BRAF and that patients with lymph node metastasis were less likely to be discordant compared to those with metastasis to other sites. Females, though not statistically significant, were also more likely to have discordant BRAF status than males, however, the cohort we studied included only patients with matched primary and metastatic melanoma lesions, making this an inherently biased sample. Factors such as patient age, anatomic location of the primary lesion and time to metastasis were not predictive of a discordant status. The importance of identifying determinants of discordance lays in the future establishment of clinical risk groups to classify subsets of patients that would benefit from retesting of their metastatic lesions before the initiation of targeted therapy.

The final section of our studies focuses of patients with multiple metastatic lesions. In our 8 patient series, we found a 100% concordance rate. Intuitively, this advocates the testing of the most accessible metastatic site to obtain accurate BRAF mutation status. Our results are in-keeping with previous inter-metastasis concordance rates of 74–95% (Edlundh-Rose et al., 2006; Omholt et al., 2003; Yancovitz et al., 2012). The slight discrepancy may be, in part, due to our small sample size available for testing. However, given that 14% of our patients with a BRAF wild-type primary were BRAF mutant in their metastasis, and assuming metastasis status is the determinant of response, then despite our results showing high inter-metastasis concordance we suggest that melanomas that have a BRAF wild-type primary be sampled in all of their metastatic sites because of the not insignificant percentage of cases showing a switch from negative to positive. Otherwise, we may be depriving these patients from a possibly lifesaving treatment modality.

Given all the above findings, the important issue remains how to work-up and treat these patients. Clinical trials addressing the response to targeted therapy in patients with discordant/concordant BRAF statuses between their primary and metastatic lesions are necessary in the future to see, if indeed, overall survival changes among different subgroups. Until we get more information regarding response to targeted therapy in metastatic BRAF discordant metastatic melanomas, patients with BRAF positive primary lesions could hypothetically benefit from this line of treatment, irrespective of the BRAF status of their metastatic sites. This is especially important given that up to two-thirds of our tested cases switched from BRAF positive to negative when they metastasized.

The topic that we are studying is fairly new with limited number of studies available. Given that these studies may ultimately culminate in recommendations for genetic testing and that treatment with Vemurafenib is associated with significant complications including squamous cell carcinoma (Boussemart et al., 2013), it may reasonable to consider that multiple centers pool their resources into a larger, unified series to better assess the mutation rates in these patients (our samples are available) and to establish clear cut recommendations for therapy before exposing patients to a drug with significant side effects.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgment

We would like to thank the Lebanese National Council for Scientific Research organization for their support, grant # CCF11232012_00018.

Please cite this article as: Saroufim, M., et al., Comparing BRAF mutation status in matched primary and metastatic cutaneous melanomas: Implications on optimized targeted therapy, Exp. Mol. Pathol. (2014), http://dx.doi.org/10.1016/j.yexmp.2014.09.008

ARTICLE IN PRESS

References

Amaria, R.N., et al., 2012. Vemurafenib: the road to personalized medicine in melanoma. Drugs Today (Barc.) 48, 109–118.
Arozarena, I., et al., 2011. Oncogenic BRAF induces melanoma cell invasion by downregulating the cGMP-specific phosphodiesterase PDE5A. Cancer Cell 19, 45–57.
Boisvert-Adamo, K., Aplin, A.E., 2008. Mutant B-RAF mediates resistance to anoikis via Bad and Bim. Oncogene 27, 3301–3312.
Boussemart, L., et al., 2013. Prospective study of cutaneous side-effects associated with the BRAF inhibitor vemurafenib: a study of 42 patients. Ann. Oncol.
Busam, K.J., et al., 2012. Immunohistochemical analysis of BRAF(V600E) expression of primary and metastatic melanoma and comparison with mutation status and melanocyte differentiation antigens of metastatic lesions. Am. J. Surg. Pathol. 37, 413–420.
Capper, D., et al., 2011. Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. Acta Neuropathol. 122, 11–19.
Cartlidge, R.A., et al., 2008. Oncogenic BRAF(V600E) inhibits BIM expression to promote melanoma cell survival. Pigment Cell Melanoma Res. 21, 534–544.
Chapman, P.B., et al., 2011. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N. Engl. J. Med. 364, 2507–2516.
Colombino, M., et al., 2012. BRAF/NRAS mutation frequencies among primary tumors and metastases in patients with melanoma. J. Clin. Oncol. 30, 2522–2529.
Curtin, J.A., et al., 2005. Distinct sets of genetic alterations in melanoma. N. Engl. J. Med. 353, 2135–2147.
Davies, H., et al., 2002. Mutations of the BRAF gene in human cancer. Nature 417, 949–954.
Edlundh-Rose, E., et al., 2006. NRAS and BRAF mutations in melanoma tumours in relation to clinical characteristics: a study based on mutation screening by pyrosequencing. Melanoma Res. 16, 471–478.
Fecher, L.A., et al., 2008. The MAPK pathway in melanoma. Curr. Opin. Oncol. 20, 183–189.
Hauschild, A., et al., 2012. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 380, 358–365.
Jemal, A., et al., 2011. Global cancer statistics. CA Cancer J. Clin. 61, 69–90.
Karram, S., et al., 2012. Predictors of BRAF mutation in melanocytic nevi: analysis across regions with different UV radiation exposure. Am. J. Dermatopathol.
Katona, T.M., et al., 2007. Genetically heterogeneous and clonally unrelated metastases may arise in patients with cutaneous melanoma. Am. J. Surg. Pathol. 31, 1029–1037.
Long, G.V., et al., 2012. Immunohistochemistry is highly sensitive and specific for the detection of V600E BRAF mutation in melanoma. Am. J. Surg. Pathol. 37, 61–65.
Maley, C.C., et al., 2006. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nat. Genet. 38, 468–473.
Menzies, A.M., et al., 2013. Intrapatient homogeneity of BRAFV600E expression in melanoma. Am. J. Surg. Pathol. 38, 377–382.
Omholt, K., et al., 2003. NRAS and BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. Clin. Cancer Res. 9, 6483–6488.
Peyssonnaux, C., Eychene, A., 2001. The Raf/MEK/ERK pathway: new concepts of activation. Biol. Cell. 93, 53–62.
Pollock, P.M., et al., 2003. High frequency of BRAF mutations in nevi. Nat. Genet. 33, 19–20.
Saroufim, M., et al., 2014a. BRAF analysis on a spectrum of melanocytic neoplasms: an epidemiological study across differing UV regions. Am. J. Dermatopathol. 36, 68–73.
Saroufim, M., et al., 2014b. BRAF mutational epidemiology in dysplastic nevi: does different solar UV radiation exposure matter? J. Eur. Acad. Dermatol. Venereol. 28, 615–625.
Shao, Y., Aplin, A.E., 2010. Akt3-mediated resistance to apoptosis in B-RAF-targeted melanoma cells. Cancer Res. 70, 6670–6681.
Shinozaki, M., et al., 2004. Incidence of BRAF oncogene mutation and clinical relevance for primary cutaneous melanomas. Clin. Cancer Res. 10, 1753–1757.
Smalley, K.S., 2003. A pivotal role for ERK in the oncogenic behaviour of malignant melanoma? Int. J. Cancer 104, 527–532.
Taniguchi, K., et al., 2008. Intratumor heterogeneity of epidermal growth factor receptor mutations in lung cancer and its correlation to the response to gefitinib. Cancer Sci. 99, 929–935.
Wan, P.T., et al., 2004. Mechanism of activation of the RAF–ERK signaling pathway by oncogenic mutations of B-RAF. Cell 116, 855–867.
Yancovitz, M., et al., 2012. Intra- and inter-tumor heterogeneity of BRAF(V600E) mutations in primary and metastatic melanoma. PLoS One 7, e29336.

UNCORRECTED PROOF

Please cite this article as: Saroufim, M., et al., Comparing BRAF mutation status in matched primary and metastatic cutaneous melanomas: Implications on optimized targeted therapy, Exp. Mol. Pathol. (2014), http://dx.doi.org/10.1016/j.yexmp.2014.09.008