



SHORT REPORT

Open Access

Lack of *SF3B1* R625 mutations in cutaneous melanoma

Bastian Schilling¹, Nicola Bielefeld¹, Antje Sucker¹, Uwe Hillen¹, Lisa Zimmer¹, Dirk Schadendorf¹, Michael Zeschnigk² and Klaus G Griewank^{1*}

Abstract

Background: Melanoma is a deadly disease affecting people worldwide. Genetic studies have identified different melanoma subtypes characterized by specific recurrently mutated genes and led to the successful clinical introduction of targeted therapies. Hotspot mutations in *SF3B1* were recently reported in uveal melanoma. Our aim was to see if these mutations also occur in cutaneous melanoma.

Findings: We analyzed a cohort of 85 cutaneous melanoma including 22 superficial spreading, 24 acral-lentiginous, 36 nodular, and 3 lentigo-maligna melanomas. Exon 14 of *SF3B1*, containing the site of recurrent mutations described in uveal melanoma, was sequenced in all samples. Additionally, *NRAS* exon 1 and 2 and *BRAF* exon 15 were sequenced in all, *KIT* exons 9, 11, 13, 17, and 18 in 30 samples. High numbers of *BRAF* and *NRAS* mutations were identified with frequencies varying according to melanoma subtype. None of the samples were found to harbor a *SF3B1* mutation.

Conclusions: We conclude that recurrent mutations in codon 625 of *SF3B1* as reported in uveal melanoma are not present in most types of cutaneous melanoma. This highlights the genetic differences between cutaneous and uveal melanoma and the need for subtype specific therapeutic approaches.

Keywords: Melanoma, *SF3B1*, Cancer genetics, Dermatology

Introduction

Malignant melanoma is a devastating disease worldwide [1,2]. Curative management of melanoma is limited to the stage of localized disease. Once metastatic spread has occurred, prognosis of patients is poor. However, a number of promising new treatment regimens have been introduced recently, showing for the first time a therapy induced increase in overall survival [3,4].

Over the last couple of decades a number of genetic alterations have been identified in melanoma. Activating driver mutations in genes such as *NRAS* [5] and *BRAF* [6] were identified in cutaneous melanoma. Losses of tumor suppressors such as *CDKN2A* and *PTEN* have been well documented [7]. In uveal melanoma a different set of genes shows recurrent mutations, including *GNAQ* and *GNA11* [8,9], with activating mutations as well as in *BAP1* [10] showing inactivating mutations. The distinct

mutation profiles of cutaneous and uveal melanoma are striking and support a model of different developmental pathways. However there is some overlap in tumor biology as ~80% of blue nevi, which are benign melanocytic tumors of the skin, also harbor *GNAQ* or *GNA11* mutations, [8] and *BAP1* mutations can be found in both cutaneous nevi and cutaneous melanoma [11-14].

Both genetic and immunohistological assays are becoming more and more relevant in determining the dignity and prognosis of melanocytic neoplasms [15-18]. Further refining which biomarkers are relevant in which settings should allow pathologists and clinicians to make more detailed diagnostic calls, leading to appropriate follow-up and treatment decisions.

Recently a recurrent mutation hotspot in *SF3B1* affecting codon 625 was found in 18.6% of uveal melanoma [19]. *SF3B1* mutations had been previously detected in myeloid malignancies such as CLL (chronic lymphoid leukemia) and MDS (myelodysplastic syndrome) [20,21] and also reported in breast cancer [22]. *SF3B1* is a splice factor, with mutations expected to result in altered pre

* Correspondence: klaus.griewank@uk-essen.de

¹Department of Dermatology, University Hospital, University Duisburg-Essen, Hufelandstrasse 55, Essen 45147, Germany

Full list of author information is available at the end of the article

mRNA splicing. However the exact target of altered splicing is unknown and might be cell type dependent [22].

The goal of our study was to analyze if *SF3B1* mutations not only play a role in uveal, but also in cutaneous melanoma.

Material and methods

Sample selection and histopathology

Cutaneous melanoma samples were obtained from the tumor bank of the Department of Dermatology, University Hospital, University Duisburg-Essen. The study was done with approval of the local ethics committee of the University of Duisburg-Essen.

DNA isolation

10 µm-thick sections were cut from formalin-fixed, paraffin-embedded tumor tissues. The sections were deparaffinized and manually microdissected according to standard procedures. Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Direct (Sanger) sequencing

Nested PCR was performed to amplify *BRAF* exon 15 and *NRAS* exon 1 and 2 and sequenced as previously described [23]. Sequencing of *KIT* exons 9, 11, 13, 17, and 18 was performed similarly. The first 120 base pairs of *SF3B1* exon 14 (covering codons 603–641) were sequenced using the forward primer – TGTTTACATTTTAGGCTG CTGGT and reverse primer – GCCAGGACTTCTTGCT TTTG. After purification with the QIAquick PCR Purification Kit (Qiagen) PCR products were used as templates for sequencing in both directions. The sequencing chromatogram files were examined, and mutations were identified using Chromas software (version 2.01, University of Sussex, Brighton, United Kingdom).

Results

Sample cohort

The cohort included tumors from 51 males and 34 females, including 22 superficial spreading, 24 acral-lentiginous, 36 nodular, and 3 lentigo-maligna melanomas, with an average

Breslow tumor thickness of 3.62 mm. The average thickness between subtypes varied; acral-lentiginous melanoma (ALM) = 4.54 mm, nodular melanoma (NM) = 4.47 mm, superficial spreading melanoma (SSM) = 1.9 mm and lentigo maligna melanoma (LMM) = 0.53 mm.

NRAS, *BRAF*, and *KIT* mutations

We analyzed 85 cutaneous melanomas in total. *BRAF* Exon 15 and *NRAS* Exon 1 and 2 were analyzed for presence of mutations by Sanger sequencing (Table 1). We identified 36 *BRAF* mutations (35 p.V600E, 1 p.V600K) and 19 *NRAS* mutations (11 p.Q61K, 3 p.Q61L, 5 p.Q61R). In total, 65% of tumors showed either a *BRAF* or *NRAS* mutation (42% *BRAF*, 22% *NRAS*). As reported previously, the mutations were found to be mutually exclusive. Prevalence of *BRAF* and *NRAS* mutations varied by histologic subtype; ALM - 33% *BRAF*, 13% *NRAS*, NM - 42% *BRAF*, 25% *NRAS*, and SSM - 60% *BRAF*, 23% *NRAS* mutations. Presence of *KIT* mutations was analyzed in 30 cases. 18 of these were in ALM in which the highest percentage of *KIT* mutations would be expected (18/24 ALM total = 75%) [24–26]. One ALM sample was found to harbor a p.N505H (c.1513A > C) mutation/variant. We further analyzed 7 NM, 4 SSM, and 1 LMM, not identifying any *KIT* mutations.

SF3B1 analysis

The first 120 base pairs of exon 14, containing the location of the known hotspot mutation at codon 625, were sequenced in all 85 samples. In four samples amplification failed or sequence reads were ambiguous. None of the remaining 81 samples showed a mutation in *SF3B1* as seen in a control sample from a uveal melanoma (Figure 1).

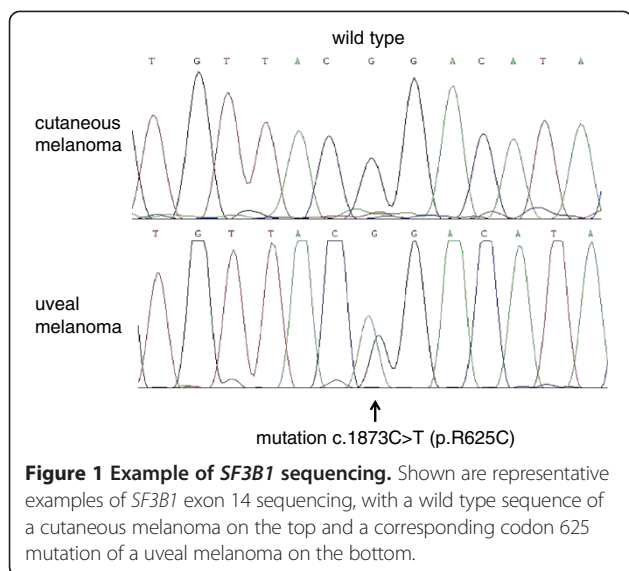
Discussion

Genetic classification of different melanoma subtypes has become very important, especially with the introduction of effective therapies targeting genetic alterations such as *BRAF* [3,4] and *KIT* mutations [25]. A detailed understanding of the genetic events occurring in different tumors will most likely prove critical to further

Table 1 Table of sequencing results

	Oncogene mutation status								SF3B1			
	Total Count	WT		BRAF V600		NRAS Q61		NA		WT		
		Count	Count	%	Count	%	Count	%	Count	%	Count	%
SSM	22	4	18.2%	13	59.1%	5	22.7%	1	4.5%	21	95.5%	
NM	36	12	33.3%	15	41.7%	9	25.0%	3	8.3%	33	91.7%	
ALM	24	13	54.2%	8	33.3%	3	12.5%	0	.0%	24	100.0%	
LMM	3	1	33.3%	0	.0%	2	66.7%	0	.0%	3	100.0%	

WT, Wildtype; SSM, Superficial spreading melanoma; NM, Nodular melanoma; ALM, Acral lentiginous melanoma; LMM, Lentigo maligna melanoma. NA, Not available (amplification failed or sequence reads were ambiguous).



improving the therapeutic modalities for metastasized melanoma patients.

The distribution of activating oncogene mutations in *BRAF* and *NRAS* in our cohort is comparable to those reported elsewhere [7]. Overall 65% of melanoma had a *BRAF* or *NRAS* mutation in a mutually exclusive pattern. Of the three melanoma subtypes analyzed in considerable numbers (SSM, NM, ALM), percentages of *BRAF* and *NRAS* mutations combined were highest in SSM reaching 82%, lower in NM with 67% and lowest in ALM with 46%. The *KIT* mutation/variant identified in an ALM sample led to a p.N505V change. This is not reported to be a frequent mutation in cutaneous melanoma [27]. However p.N505H (c.1513A > C) is listed as a “variant of unknown origin” in a gastrointestinal stromal tumor in the COSMIC database [28]. The cutaneous ALM sample lacked mutations in *BRAF* or *NRAS* which could support a potential relevance, as typically *KIT* mutations are found to be mutually exclusive with *BRAF* and *NRAS* mutations [26]. The p.N505H (c.1513A > C) change could however also represent a rare germ-line variant, which we could not check as corresponding normal DNA was not available.

We obtained high quality sequencing results allowing analysis of exon 14 and in particular codon 625 of *SF3B1* in 81 samples and found no mutations. This argues against a major role for *SF3B1* in tumorigenesis or progression of cutaneous melanoma. In uveal melanomas, mutations were primarily found in tumors with a favorable prognosis [19]. Future studies could analyze if *SF3B1* mutations occur in benign cutaneous melanocytic tumors (nevi) or potentially in sites other than in codon 603–641 of exon 14 of *SF3B1*.

In recent years genetic analyses identified a number of key genes involved in melanoma formation or progression.

Interestingly, almost all of those described in cutaneous melanoma are not known to be relevant in uveal melanoma [29,30]. In contrast, genetic alterations in uveal melanoma such as *GNAQ* and *GNA11* mutations were also found in selected cases of cutaneous melanoma and are frequently found in blue nevi (benign cutaneous melanocytic tumors) [9]. *BAP1* inactivating mutations are found in cutaneous nevi and melanoma, although considerably less frequently than in uveal melanoma [12]. Our current study would signify that *SF3B1* mutations, occurring in almost 20% of uveal melanoma, [19] do not play a major role in cutaneous melanoma. We believe this highlights once more the genetic differences between uveal and cutaneous melanoma and the need for development of melanoma subtype specific therapies.

Abbreviations

ALM: Acral-lentiginous melanoma; SSM: Superficial spreading melanoma; NM: Nodular melanoma; LMM: Lentigo maligna melanoma.

Competing interests

Dirk Schadendorf is on the advisory board or has received honoraria from Roche, Genetech, Novartis, Amgen, GSK, BMS, Boehringer Ingelheim, and Merck. All other authors have nothing to declare.

Authors' contributions

Literature search: BS, LZ, MZ, DS, KGG Study design: BS, AS, DS, MZ, KGG Data collection: NB, AS, BS, UH, KGG Data analysis: BS, NB, AS, KGG Data interpretation: BS, LZ, DS, MZ, UH KGG Manuscript writing: all authors. All authors read and approved the final manuscript.

Acknowledgments

We would like to thank Iris Moll, Sabine Prass, and Marion Schwamborn for their excellent technical support.

Financial disclosure

The research was supported by a grant from the MERCUR-Stiftung. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author details

¹Department of Dermatology, University Hospital, University Duisburg-Essen, Hufelandstrasse 55, Essen 45147, Germany. ²Department of Human Genetics, University Hospital, University Duisburg-Essen, Hufelandstrasse 55, Essen 45147, Germany.

Received: 4 April 2013 Accepted: 16 May 2013

Published: 21 May 2013

References

1. Siegel R, Naishadham D, Jemal A: **Cancer statistics, 2012.** *CA Cancer J Clin* 2012, **62**:10–29.
2. Flaherty KT, Hodi FS, Fisher DE: **From genes to drugs: targeted strategies for melanoma.** *Nat Rev Cancer* 2012, **12**:349–361.
3. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, et al: **Improved survival with vemurafenib in melanoma with BRAF V600E mutation.** *N Engl J Med* 2011, **364**:2507–2516.
4. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, Hamid O, Schuchter L, Cebon J, Ibrahim N, et al: **Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations.** *N Engl J Med* 2012, **367**:1694–1703.
5. Ball NJ, Yohn JJ, Morelli JG, Norris DA, Golitz LE, Hoeffler JP: **Ras mutations in human melanoma: a marker of malignant progression.** *J Invest Dermatol* 1994, **102**:285–290.

6. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, *et al*: **Mutations of the BRAF gene in human cancer.** *Nature* 2002, **417**:949–954.
7. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho KH, Aiba S, Brocker EB, LeBoit PE, *et al*: **Distinct sets of genetic alterations in melanoma.** *N Engl J Med* 2005, **353**:2135–2147.
8. Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, Simpson EM, Barsh GS, Bastian BC: **Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi.** *Nature* 2009, **457**:599–602.
9. Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, Obenaus AC, Wackernagel W, Green G, Bouvier N, *et al*: **Mutations in GNA11 in uveal melanoma.** *N Engl J Med* 2010, **363**:2191–2199.
10. Harbour JW, Onken MD, Roberson ED, Duan S, Cao L, Worley LA, Council ML, Matattal KA, Helms C, Bowcock AM: **Frequent mutation of BAP1 in metastasizing uveal melanomas.** *Science* 2010, **330**:1410–1413.
11. Wiesner T, Murali R, Fried I, Cerroni L, Busam K, Kutzner H, Bastian BC: **A distinct subset of atypical Spitz tumors is characterized by BRAF mutation and loss of BAP1 expression.** *Am J Surg Pathol* 2012, **36**:818–830.
12. Wiesner T, Obenaus AC, Murali R, Fried I, Griewank KG, Ulz P, Windpassinger C, Wackernagel W, Loy S, Wolf I, *et al*: **Germline mutations in BAP1 predispose to melanocytic tumors.** *Nat Gen* 2011, **43**:1018–1021.
13. Carbone M, Ferris LK, Baumann F, Napolitano A, Lum CA, Flores EG, Gaudino G, Powers A, Bryant-Greenwood P, Krausz T, *et al*: **BAP1 cancer syndrome: malignant mesothelioma, uveal and cutaneous melanoma, and MBAITs.** *J Trans Med* 2012, **10**:179.
14. Njauw CN, Kim I, Piris A, Gabree M, Taylor M, Lane AM, DeAngelis MM, Gragoudas E, Duncan LM, Tsao H: **Germline BAP1 inactivation is preferentially associated with metastatic ocular melanoma and cutaneous-ocular melanoma families.** *PLoS One* 2012, **7**:e35295.
15. Moore MW, Gasparini R: **FISH as an effective diagnostic tool for the management of challenging melanocytic lesions.** *Diagn Pathol* 2011, **6**:76.
16. Bauer J, Bastian BC: **Distinguishing melanocytic nevi from melanoma by DNA copy number changes: comparative genomic hybridization as a research and diagnostic tool.** *Dermatol Ther* 2006, **19**:40–49.
17. Nodin B, Fridberg M, Jonsson L, Bergman J, Uhlen M, Jirstrom K: **High MCM3 expression is an independent biomarker of poor prognosis and correlates with reduced RBM3 expression in a prospective cohort of malignant melanoma.** *Diagn Pathol* 2012, **7**:82.
18. Schimming TT, Grabellus F, Roner M, Pechlivanis S, Sucker A, Bielefeld N, Moll I, Schadendorf D, Hillen U: **pHH3 immunostaining improves interobserver agreement of mitotic index in thin melanomas.** *Am J Dermatopathol* 2012, **34**:266–269.
19. Harbour JW, Roberson ED, Anbunathan H, Onken MD, Worley LA, Bowcock AM: **Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma.** *Nat Gen* 2013, **45**(2):133–135. doi:10.1038/ng.2523. Epub 2013 Jan 13.
20. Papaemmanuil E, Cazzola M, Boultonwood J, Malcovati L, Vyas P, Bowen D, Pellagatti A, Wainscoat JS, Hellstrom-Lindberg E, Gambacorti-Passerini C, *et al*: **Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts.** *N Engl J Med* 2011, **365**:1384–1395.
21. Quesada V, Conde L, Villamor N, Ordonez GR, Jares P, Bassaganyas L, Ramsay AJ, Bea S, Pinyol M, Martinez-Trillos A, *et al*: **Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia.** *Nat Gen* 2012, **44**:47–52.
22. Ellis MJ, Ding L, Shen D, Luo J, Suman VJ, Wallis JW, Van Tine BA, Hoog J, Goiffon RJ, Goldstein TC, *et al*: **Whole-genome analysis informs breast cancer response to aromatase inhibition.** *Nature* 2012, **486**:353–360.
23. Houben R, Becker JC, Kappel A, Terheyden P, Brocker EB, Goetz R, Rapp UR: **Constitutive activation of the Ras-Raf signaling pathway in metastatic melanoma is associated with poor prognosis.** *J Carcinogen* 2004, **3**:6.
24. Beadling C, Jacobson-Dunlop E, Hodi FS, Le C, Warrick A, Patterson J, Town A, Harlow A, Cruz F 3rd, Azar S, *et al*: **KIT gene mutations and copy number in melanoma subtypes.** *Clin Cancer Res J Am Assoc Cancer Res* 2008, **14**:6821–6828.
25. Carvajal RD, Antonescu CR, Wolchok JD, Chapman PB, Roman RA, Teitcher J, Panageas KS, Busam KJ, Chmielowski B, Lutzky J, *et al*: **KIT as a therapeutic target in metastatic melanoma.** *JAMA* 2011, **305**:2327–2334.
26. Curtin JA, Busam K, Pinkel D, Bastian BC: **Somatic activation of KIT in distinct subtypes of melanoma.** *J Clin Oncol J Am Soc Clin Oncol* 2006, **24**:4340–4346.
27. Garrido MC, Bastian BC: **KIT as a therapeutic target in melanoma.** *J Invest Dermatol* 2010, **130**:20–27.
28. Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, Beare D, Jia M, Shepherd R, Leung K, Menzies A, *et al*: **COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer.** *Nucleic Acids Res* 2011, **39**:D945–950.
29. Krauthammer M, Kong Y, Ha BH, Evans P, Bacchicocchi A, McCusker JP, Cheng E, Davis MJ, Goh G, Choi M, *et al*: **Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma.** *Nat Gen* 2012, **44**:1006–1014.
30. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, Nickerson E, Auclair D, Li L, Place C, *et al*: **A landscape of driver mutations in melanoma.** *Cell* 2012, **150**:251–263.

doi:10.1186/1746-1596-8-87

Cite this article as: Schilling *et al*: **Lack of SF3B1 R625 mutations in cutaneous melanoma.** *Diagnostic Pathology* 2013 **8**:87.

Submit your next manuscript to BioMed Central and take full advantage of:

- **Convenient online submission**
- **Thorough peer review**
- **No space constraints or color figure charges**
- **Immediate publication on acceptance**
- **Inclusion in PubMed, CAS, Scopus and Google Scholar**
- **Research which is freely available for redistribution**

Submit your manuscript at
www.biomedcentral.com/submit

