Identification of novel targetable mutations in metastatic anorectal melanoma by next-generation sequencing

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INTRODUCTION

The molecular classification of melanomas, which can have diverse clinical and histopathologic features, is defined by the acquisition of somatic mutations. Mutations such as *BRAF* V600E result in constitutive activation of critical signaling pathways that promote formation of melanocytic nevi.[1] Acquisition of subsequent mutations induces the progression to melanomagenesis, and further accumulation of tertiary mutations might promote metastasis. These molecular pathways remain largely undiscovered. Here we describe the use of the Stanford solid tumor actionable mutation panel (STAMP), a targeted next-generation sequencing (NGS) panel comprising 130 genes selected on the basis of their known impact as actionable targets of existing and emerging anti-cancer therapies, prognostic features, and mutation recurrence frequency across patients with known cancer types, including melanoma.

CASE REPORT

A 75-year-old man with stage III anorectal mucosal melanoma had a lung metastasis, and both lesions were subjected to STAMP. The primary lesion was initially diagnosed during a hemorrhoidectomy procedure and consisted of a 1.2 × 0.6−cm polypoid, ulcerating mass with histologic findings of diffusely atypical melanocytes (Fig 1). The results of NGS demonstrated that the primary and metastatic lesion were identical with the exception of 1 detectable mutation in *PTEN* c.892C>T, p. Gln298Ter (Table I), resulting in a truncation. *PTEN* has previously been identified as a gene that might have mutations present in advanced and metastatic cutaneous melanoma but has not been described in anorectal melanoma.[1,2]

Abbreviations used:

NGS: next-generation sequencing
STAMP: Stanford targeted next-generation sequencing panel

DISCUSSION

In a series of anorectal melanomas, Yang et al[2] found anorectal melanomas have driver mutations in *KIT*, *NF1*, *SF3B1*, *TP53*, *HRAS*, *BRAF*, and *MLH1*. In a separate study of cutaneous melanoma, Shain et al[1] proposed a model for the progression of cutaneous melanomas from benign nevi and found common driver mutations in *BRAF*, *NRAS*, *TERT*, *CDKN2A*, *NF1*, *HRAS*, and the ARID gene family with *TP53* and *PTEN* mutations identified in more advanced to metastatic melanoma. Through NGS, we identified a mutation in metastatic anorectal melanoma, supporting a potential global role of *PTEN* in promoting melanoma metastasis.

In addition, 5 variants of unknown significance (*BCR* p.Val949Ile, *BRCA1* p.Arg841Trp, *MET* c.2941+49T>G, *NFE2L2* p.Leu266Phe, and *PTCH1* p.Gly15_Gly17del) were identified in both primary and metastatic lesions (Table I). Overexpression of *MET* has previously been associated with metastatic melanoma, and this association was replicated in mouse models.[3] Mutations in *BRCA1* and *PTCH1*

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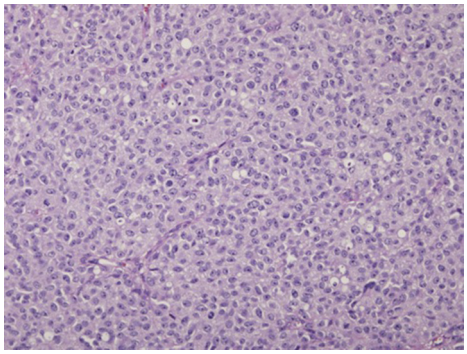

Fig 1. Invasive anorectal melanoma. Anorectal invasive melanoma demonstrates a diffuse proliferation of atypical melanocytes with abundant amphophilic cytoplasm and nuclear polymorphism. (Hematoxylin-eosin stain; original magnification: ×200.)

Table I. Mutations in stage III primary mucosal anorectal melanoma and metastatic lung melanoma identified by next-generation sequencing of targeted and actionable mutations

Gene	Variant
Known pathogenic significance	
*PTEN**	p.Gln298Ter
NF1	p.Met442fs
SF3B1	p.Arg625His
Unknown significance	
BCR	p.Val949Ile
BRCA1	p.Arg841Trp
MET	c.2941+49T>G
NFE2L2	p.Leu266Phe
PTCH1	p.Gly15_Gly17del

*Mutation present in metastatic lesion only.

have been previously described in melanoma but without detailed investigation of their role in the progression from benign nevi to melanoma and metastasis.[4] Higher *NFE2L2* expression has been associated with worse prognosis in melanoma,[5] but analysis of novel *NFE2L2* mutations in melanoma has not been conducted. There are no previous reports on the association between mutations in *BCR* and melanoma. The identification of additional mutations in metastasis supports the role of NGS to guide personalized therapy for advanced lesions.

Targeted NGS presents a new opportunity to expand existing knowledge of mutations for various subtypes of melanoma to further define the mechanisms of initiation and progression. This case identifies a mutation, *PTEN* p. Gln298Ter (ie, unique to the metastatic site), which might represent the primary driver mutation for melanoma metastasis in this patient. Biopsies of additional metastatic sites were not available in this case; however, identification of *PTEN* p. Gln298Ter at additional sites would provide strong evidence for the role of *PTEN* p. Gln298Ter in the development of distal metastasis. This would further support the role of *PTEN* in driving metastasis in both primary cutaneous and anorectal melanomas.

In the clinical management of the patient in this case, the results of the STAMP panel provided guidance to continue the patient on targeted immunotherapies other than *BRAF* inhibitors. Current targeted treatment of advanced stage melanoma is primarily dependent on *BRAF* mutation status.[6] In addition, *PTEN* inactivation has previously been noted to significantly shorten overall survival and time to metastasis to brain and liver in patients with *BRAF* V600 mutations, as well as increase resistance to *BRAF* inhibitor therapy.[7] However, in patients without *BRAF* V600 mutations, there was not a significant association between *PTEN* inactivation and time to brain metastasis or length of overall survival. This *BRAF* mutation−negative, *PTEN* p. Gln298Ter patient was started on pembrolizumab, a programmed cell death 1 inhibitor and a first-line therapy for advanced stage melanoma. This patient was also found to harbor a pathogenic mutation in *NF1*; however, this patient was not enrolled in any experimental *NF1*-targeted therapies.

This case additionally presents variants in *BCR, BCRA1, NFE2L2, PTCH1,* and *MET* that require further investigation to ascertain their role in melanomagenesis and progression, as well as their clinical significance for disease prognosis and treatment. Thus, the study of these new variants holds the promise of promoting the identification of additional drug targets for the treatment of melanoma.

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REFERENCES

1. Shain AH, Yeh I, Kovalyshyn I, et al. The genetic evolution of melanoma from precursor lesions. *New Engl J Med*. 2015; 373(20):1926-1936.
2. Yang HM, Hsiao SJ, Schaeffer DF, et al. Identification of recurrent mutational events in anorectal melanoma. *Mod Pathol*. 2017;30(2):286-296.
3. Otsuka T, Takayama H, Sharp R, et al. c-Met autocrine activation induces development of malignant melanoma and acquisition of the metastatic phenotype. *Cancer Res*. 1998; 58(22):5157.
4. Monnerat C, Chompret A, Kannengiesser C, et al. *BRCA1, BRCA2, TP53*, and *CDKN2A* germline mutations in patients with breast cancer and cutaneous melanoma. *Fam Cancer*. 2007; 6(4):453-461.
5. Hintsala H-R, Haapasaari K-M, Soini Y, Karihtala P. An immunohistochemical study of NFE2L2, KEAP1 and 8-hydroxy-2′-deoxyguanosine and the EMT markers SNAI2, ZEB1 and

TWIST1 in metastatic melanoma. *Histol Histopathol*. 2017;32(2):129-136.

6. Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: optimizing outcomes in melanoma. *Nat Rev Clin Oncol*. 2017;14(8):463-482.
7. Bucheit AD, Chen G, Siroy A, et al. Complete loss of PTEN protein expression correlates with shorter time to brain metastasis and survival in stage IIIB/C melanoma patients with *BRAF* V600 mutations. *Clin Cancer Res*. 2014;20(21):5527.