Spotlight on Clinical Response

Intratumoral Molecular Heterogeneity in a BRAF-Mutant, BRAF Inhibitor-Resistant Melanoma: A Case Illustrating the Challenges for Personalized Medicine

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Abstract

Targeted therapies are increasingly being used to treat a variety of cancers. Their efficacy depends upon the accurate detection and targeting of a specific mutation or aberration in the tumor. All cancers, such as melanoma, are molecularly heterogeneous, with drug-resistant subclones present before the treatment or emerging as a result of targeted therapies. Here, we show intraleSIONal molecular heterogeneity in a progressing V600E BRAF-mutant melanoma metastasis from a patient treated for 7 months with the BRAF inhibitor vemurafenib. In the single metastasis, two distinct subclones were observed, both V600E BRAF-mutant and only one with an additional G13R NRAS mutation. Molecular heterogeneity even at the intraleSIONal level shows that personalizing or adjusting therapies based on genotyping of a portion of a single lesion may not accurately depict the molecular profile or drivers of oncogenesis across the entire patient’s melanoma. Mol Cancer Ther; 11(12), 2704–8. ©2012 AACR.

Introduction

New tumor mutation–specific targeted therapies are revolutionizing the treatment of many solid tumors (1) including melanoma (2, 3). However, tumor molecular heterogeneity is rapidly emerging as a complicating factor in the efficacy of targeted therapies, the durability of responses, and the development of drug resistance. The selective pressure induced by targeted therapies can result in the dominance or acquisition of additional driver mutations or molecular aberrations in tumor subclones. Constitutive activating mutations of the B-raf (BRAF) gene occur in approximately half of all patients with metastatic melanoma (3). BRAF inhibitors have proven highly active (3) and improve overall survival compared with DTIC in patients with BRAF mutant metastatic melanoma (2). Eventually, most patients develop resistance to BRAF inhibition and relapse. Multiple mechanisms of resistance have been elucidated (4), including the reactivation of the MAPK pathway through the development of an NRAS mutation (5). Minority NRAS mutant subclones may preexist BRAF inhibitor treatment or the BRAF mutant population may acquire a secondary NRAS mutation after BRAF inhibitor exposure. This study shows tumor heterogeneity exists within a BRAF inhibitor–resistant lesion, which has implications for biopsy driven personalized medicine.

Case report

A 71-year-old man presented in February 2009 with a paraumbilical subcutaneous melanoma metastasis. He had a past history of a primary cutaneous melanoma of the right lower leg diagnosed in April 2005 and a right groin recurrence in May 2007. By May 2009, he had multiple distant melanoma metastases involving the subcutis, lung, left axilla, and adrenal glands and an isolated 6 mm brain metastasis. The patient’s paraumbilical metastasis was tested for genomic mutations within exon 15 of the BRAF gene using high resolution melt and Sanger sequencing, revealing the presence of a BRAF V600E point mutation. He was treated with stereotactic radiosurgery to the brain metastasis, progressed after 3 months of stable disease on temozolomide +/- ABT-888/placebo (Trial registration ID: NCT00804908), and was then enrolled on the phase II study of vemurafenib, BRIM2 (6). He commenced vemurafenib (960 mg twice daily) on December 2, 2009 and achieved a RECIST (response evaluation criteria in solid tumors) defined partial response (7),

References

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doi: 10.1158/1535-7163.MCT-12-0530

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but progressed on June 29, 2010, both clinically and radiographically on computed tomography scanning (Fig. 1). Excision biopsy of subcutaneous metastases was conducted one day before the commencement of vemurafenib, on day 14 of treatment, and again on disease progression on July 20, 2010 as part of the Treat Excise Analyze for Melanoma Study at the Melanoma Institute Australia (Sydney, Australia) as approved by the Royal Prince Alfred Hospital Research Ethics Committee Protocol Nos. X10-0305 and HREC/10/RPAH. His subsequent systemic therapies included single-agent trametinib (previously GSK1120212, an MEK inhibitor; ref. 8), the combination of dabrafenib (previously GSK2118436, a BRAF inhibitor) and trametinib (9) and E7080 (a multikinase inhibitor with specific activity against VEGF), and finally he was rechallenged with vemurafenib (Fig. 1A). For each systemic therapy, excision biopsy of a melanoma metastasis was conducted on days 4 to 7 following therapy commencement and on RECIST progression, providing multiple independent tumor samples for analysis and comparison.

Results and Discussion

We sought to determine whether the patient’s initial vemurafenib resistance developed through the reactivation of the MAPK pathway due to additional mutations to the NRAS or BRAF genes. Sanger sequencing was conducted on the patient’s vemurafenib-resistant metastasis to detect somatic mutations in exon 1 and exon 2 of the NRAS gene and in exon 11 and exon 15 of the BRAF gene. This revealed a G13R NRAS mutation in addition to the original BRAF V600E mutation within the single vemurafenib-resistant metastasis (Fig. 1B–D). We used immunocytochemistry to analyze the expression levels of downstream signaling proteins within the progression biopsy to identify any subclones of tumor cells within the lesion. Immunohistochemistry was conducted on the progressed metastasis using antibodies against Ki-67, p-ERK1/2, p-AKT, cyclin D1, MITF, p27Kip1, p16INK4a, p53, BCL-2, Mcl-1, PTEN, MAP3K8 (COT), IGF1Rβ, and PDGFRβ. The staining for p-ERK1/2 expression clearly identified 2 areas of tumor with differential staining (Fig. 2A). The percentage of immunopositive tumor
cells was assessed for each subclone using a Dako ACIS III Automated Image Analysis System. One subclone had high p-ERK1/2 expression (95% tumor cells positive, labeled subclone A; Fig. 2D), and was adjacent to melanoma cells with low expression of p-ERK1/2 (3% tumor cells positive, labeled subclone B; Fig. 2E). The proliferative rate represented by Ki-67 expression was higher in the subclone A (10% tumor cells positive) than in subclone B (3% tumor cells positive; Fig. 2F and G). Conversely, the percentage of cyclin D-positive tumor cells was lower in subclone A than in subclone B (46% and 74%, respectively; Fig. 2H and I). The expression of the remaining proteins did not seem to differ between the subclones.

We then carried out mutation testing separately on each of the subclones A and B, which were identified by their p-ERK1/2 expression. Each subclone was macrodissected and genomic DNA was extracted from each individual.
subclone. *BRAF* and *NRAS* mutation status of the subclones was determined by allele-specific specific real-time PCR (RT-PCR). In addition, subclone genomic DNA was analyzed by Mass Spectrometric SNP genotyping with OncoCarta v0.1 and the MelaCarta panel v0.1 to detect any additional somatic mutations. The genomic DNA mutational status of subclones A and B was different; subclone A was *NRAS* wild-type and *BRAF*V600E mutant (Fig. 2B), whereas subclone B was *NRAS* G13R mutant and *BRAF*V600E mutant (Fig. 2C). The sensitivity of this allele-specific RT-PCR allowed the detection of as little as 2% mutant *NRAS* and *BRAF* allele (data not shown; ref. 10). For this reason, it is unlikely that the differential mutational status of the subclones is due to the assay sensitivity. The OncoCarta and MelaCarta did not reveal any additional mutations.

Mutational analysis using mutant-specific RT-PCR of the antecedent primary melanoma and lymph node metastases that occurred 5 and 3 years before the diagnosis of widely disseminated metastatic disease, revealed the presence of V600E *BRAF* mutation, but not the *NRAS* G13R mutation (Fig. 1A). Similarly, analysis of 7 metastatic tumors resected after cessation of treatment with vemurafenib and during treatment with single-agent trama
tinib or the combination of trametinib and dabrafenib detected a *BRAF*V600E mutation without a *NRAS*G13R mutation in all biopsies (Fig. 1A).

Here, we show intratumoral molecular heterogeneity in a progressing V600E *BRAF*-mutant melanoma metastasis from a patient treated for 7 months with the *BRAF* inhibitor vemurafenib. Within the same individual progressing and vemurafenib-resistant metastasis, 2 distinct subpopulation were observed, both V600E *BRAF*-mutant, and only one G13R *NRAS*-mutant. The acquisition of an additional *NRAS* mutation causing MAPK pathway reactivation has been proposed as a mechanism of *BRAF* inhibitor resistance in *BRAF* mutant melanoma patients (5). In this study, the *NRAS* G13R mutation may be one but not the sole driver of disease progression. This issue of tumor heterogeneity is further complicated by incomplete tumor shrinkage, at the macroscopic and radiologic levels, induced by *BRAF* inhibitors in the majority of tumors and patients. Thus, a biopsy of tumor progression on any *BRAF* inhibitor may frequently yield a mixture of tumor cells with distinct kinetic growth properties. That an *NRAS* mutation was not detected in the subsequent tumor biopsies raises tantalizing questions as to how the MEK inhibitor treatment alone (first sequential therapy after failure on vemurafenib alone) might have influenced the selective pressure, for or against, the double *BRAF/NRAS* mutant melanoma population. Our tissue biopsy series here illustrate the dynamic nature of tumor evolution on multiple distinct targeted regimens.

In this patient, we have shown that intratraselographic genetic heterogeneity is present within *BRAF* inhibitor-resistant metastatic melanoma and highlighted the fact that sampling error is a potential pitfall of fine-needle aspiration or punch biopsy techniques. Advances in genome analysis methods such as deep sequencing (11–13) and sequencing of circulating tumor cells (14) may provide superior information regarding the heterogeneity of a patient’s tumors. These methods remain exploratory due in part to the complex analyses required. Knowledge of heterogeneity may help predict and improve a patient’s response to combination therapies based on the level and signature of mutational heterogeneity. These technologies may also help confront another likely issue arising from the tumor heterogeneity: multiple mechanisms of acquired drug resistance in the same progressing tumor or multiple progressing tumors in the same patient.

However, the current method of assessing heterogeneity in a subset of sampled lesions is unlikely to adequately predict tumor heterogeneity *in vivo*, nor reflect the ongoing genetic changes that occur during treatment (13, 15). Thus, the strategy of biopsying metastatic disease to decide the next systemic therapy after progression on a targeted therapy will be complicated by heterogeneity. We need to anticipate multiple mechanisms of resistance, predict the critical downstream effector pathways, and treat with the most effective broad-ranging combination of therapies at first diagnosis of metastatic disease. These therapies may include the targeting of oncogenes along with immunotherapy or chemotherapy (16–18).

**Disclosure of Potential Conflicts of Interest**

R.F. Kefford is a consultant/advisory board member and has a hono- raria and travel support from GlaxoSmithKline. G.V. Long is a consultant/advisory board member in Roche, Bristol-Myers Squibb, and GlaxoSmithKline and has a honoraria from Roche and Merck and travel support from GlaxoSmithKline and Roche. G.V. Long also has a commerc- ial research support from Roche. R.A. Scolyer is a consultant/advisory board member of Roche and GlaxoSmithKline and has a honoraria from Abbott Molecular and Roche. J.F. Thompson has a honoraria and is a consultant/advisory board member of Roche and GlaxoSmithKline. G.V. Long and R.A. Scolyer are funded by the Cancer Institute New South Wales Fellowship program. H. Rizos is a recipient of a Cancer Institute New South Wales Research Fellowship and an NHMRC Senior Research Fellowship. The funding body had no role in the design or conduct of the study. No potential conflicts of interest were disclosed by the other authors.

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**Grant Support**

This work is supported by the Program Grant 402761 of the National Health and Medical Research Council of Australia (NHMRC), Translational Research Program Grant 08/TPG/1-01 of the Cancer Institute New
South Wales (NSW), and an infrastructure grant to Westmead Millennium Institute by the Health Department of NSW through Sydney West Area Health Service. Westmead Institute for Cancer Research is the recipient of capital grant funding from the Australian Cancer Research Foundation.

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Received May 28, 2012; revised August 26, 2012; accepted August 31, 2012; published OnlineFirst September 7, 2012.

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