8-1-2013

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Edward J Hartsough
Thomas Jefferson University

A E Aplin
Thomas Jefferson University

Published in final edited form as:
J Invest Dermatol. 2013 August ; 133(8): 1928–1929. doi:10.1038/jid.2013.136.

A STATement on Vemurafenib-Resistant Melanoma

Edward J. Hartsough and **Andrew E. Aplin**
Department of Cancer Biology and Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107

Edward J. Hartsough: ejh004@Jefferson.edu; Andrew E. Aplin: Andrew.Aplin@Jefferson.edu

Summary

Despite recent advancements in the treatment of late-stage mutant BRAF $^{V600E/K}$ melanomas, a major hurdle continues to be acquired resistance to BRAF inhibitors such as Vemurafenib. The mechanisms for resistance have proven to be heterogeneous, emphasizing the need to utilize broad therapeutic approachs. The present study, “Stat3 targeted therapies overcome the acquired resistance to vemurafenib in melanomas” by Liu et al., proposes that STAT3-PAX3 signaling may be a mechanism that is utilized by melanomas to resist RAF inhibitors.

Mutations in the serine/threonine kinase BRAF are found in 45–50% of melanomas. The development of clinical inhibitors to steps in the BRAF-MEK-ERK1/2 pathway have led to FDA-approval of the RAF inhibitor vemurafenib in late-stage mutant BRAF $^{V600E/K}$ melanomas. However, the initial clinical responses to vemurafenib are heterogeneous, with median progression-free survival of only 6–7 months. Almost all patients who experience an initial response ultimately acquire resistance that allows disease progression, emphasizing the need to identify and target mechanisms of resistance both to vemurafenib and to other RAF inhibitors. Several mechanisms employed by mutant BRAF melanoma cells to overcome RAF inhibition have been described previously (Aplin *et al.*, 2011). Most of these mechanisms, such as expression of *BRAF* splice variants, COT1 expression, and activating MEK1 or NRAS mutations, funnel through the ERK1/2 pathway and lead to its re-activation. In this paper, Liu *et al.* implicate an alternative signaling pathway, signal transducer and activator of transcription 3 (STAT3)–paired box 3 (PAX3), in RAF inhibitor-resistance in melanoma.

STAT3 is a cytokine-regulated transcription factor activated by Janus kinases (JAKs), a family of non-receptor tyrosine kinases. JAKs phosphorylate STAT3 directly, inducing their dimerization and subsequent nuclear translocation. Recent work from the Cui laboratory has demonstrated STAT3 as a direct transactivator of the PAX3 promoter (Dong *et al.*, 2012). PAX3, a transcription factor belonging to the paired class homeodomain family, has been implicated in activating expression of the receptor tyrosine kinase MET in melanoma (Mascarenhas *et al.*, 2010). In the current study, Liu and colleagues show that mutant V600E BRAF enhances STAT3 phosphorylation and increases PAX3 expression in melanocytes. Targeting mutant BRAF signaling with vemurafenib inhibits STAT3 phosphorylation and expression of PAX3; this effect is attenuated but not eliminated completely in vemurafenib-resistant melanoma cells. These data seemingly oppose previous findings that inhibition of MEK, the downstream target of BRAF, leads to increased STAT3 phosphorylation (Krasilnikov *et al.*, 2003).

Nevertheless, a striking observation is that over-expression of a constitutively active form of STAT3 or wild-type PAX3 renders mutant BRAF melanoma cells more resistant to vemurafenib *in vitro*. An interesting future avenue would be to address mechanistically how PAX3 contributes to the resistant phenotype. Additionally, these results await support from vemurafenib-treated patient samples to determine whether PAX3 expression correlates with

responses. Another aspect of the work is the extrapolation from previous studies demonstrating that secreted basic fibroblast growth factor 2 (bFGF2) derived from keratinocytes activates STAT3 in melanocytic cells (Dong *et al.*, 2012). The authors hypothesize that bFGF2 secreted by melanoma cells as well as keratinocytes and fibroblasts in the tumor microenvironment ultimately lead to PAX3 expression. In the present study, Liu and colleagues were able to detect elevated levels of secreted bFGF2 in media of vemurafenib resistant cells when compared to their sensitive parental counterpart. Additionally, the authors demonstrate that bFGF2 secretion in human primary fibroblasts and human primary keratinocytes is enhanced in a dose dependent manner when exposed to vemurafenib. These data suggest that bFGF2 secretion is elicited via an unknown mechanism as a "protective" measure against vemurafenib treatment. Future studies may be able to link this vemurafenib induced bFGF2 secretion observed from cells present in the tumor micro-environment to STAT3 signaling and BRAF inhibitor resistance. Indeed, there is increasing evidence that stromal-derived factors modulate responses to RAF inhibitors (Straussman *et al.*, 2012).

Liu *et al.* highlight the importance of the STAT3-PAX3 signaling axis using knockdown experiments and WP1066, a STAT3 inhibitor. Knockdown of either STAT3 or PAX3 in vemurafenib resistant cells reduced growth significantly. Furthermore, STAT3 knockdown enhanced cell's susceptibility to vemurafenib substantially, yielding proof of principal for pre-clinical examination of WP1066, a small molecule STAT3 inhibitor. Earlier work with WP1066 has demonstrated its ability to block phosphorylation of JAK2 and STAT3, reduce melanoma proliferation, and diminish tumor growth *in vivo* (Kong *et al.*, 2008). In the present study, WP1066 inhibited phosphorylation of STAT3 and reduced downstream levels of PAX3, irrespective of vemurafenib sensitivity status. In addition, combined treatment with vemurafenib and WP1066 decreased the number of vemurafenib-resistant cells more effectively than either drug alone. While the current work has yet to determine whether there is mechanistic cooperation between V600E BRAF inhibition though vemurafenib and WP1066 elicited reduction in activated STAT3, it suggests that STAT3 targeting in melanoma may be effective. Dosing curves of these drugs in conjunction with either knockdown or overexpression studies may provide better insight into potential synergies. Because STAT3 signaling seems to be a necessary pathway for melanoma cell viability, these findings have translational implications as they may provide a broad therapeutic strategy for targeting the heterogeneity of vemurafenib-resistance mechanisms, akin to the notion recently proposed for HSP90 inhibitors (Paraiso *et al.*, 2012). Although STAT3 inhibitors such as WP1066 have yet to be evaluated fully in the clinic, JAK2/STAT3 inhibitors are currently in Phase I/II clinical trials for head and neck tumors and lymphomas. The present study lays a foundation for additional preclinical studies on the use of WP1066 and other STAT3 inhibitors in patients with vemurafenib-resistant melanomas.

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Clinical implications

1. STAT3 activity and PAX3 levels may modulate responses to RAF inhibitors in mutant BRAF melanomas.
2. STAT3 inhibitors are currently being tested in clinical trials.
3. Both autocrine and paracrine mechanisms may regulate STAT3-PAX3 signaling in response to RAF inhibitors.