Editorial

Molecular Cancer Therapeutics

Adaptive Resistance to Chemotherapy, A Multi–FAK-torial Linkage

Kristin N. Taylor and David D. Schlaepfer

Abstract

Oncogenes provide tumor cells with a growth and survival advantage. Directed therapies targeted to oncogenic mutations (such as *BRAF* V600E) are part of effective late-stage melanoma treatment. However, tumors with *BRAF* V600E mutations, in approximately 10% of colorectal cancer, are generally treatment-insensitive. Research has identified various "feedback" mechanisms that result in BRAF signal pathway reactivation in response to BRAF inhibition. Herein, we highlight key findings from Chen and colleagues (this issue) showing that integrin-associated focal adhesion kinase (FAK) activation selectively occurs in *BRAF* V600E-mutant colorectal cancer cells in response to pharmacological BRAF inhibition. FAK activation results in elevated β-catenin protein levels, β-catenin nuclear localization, and increased gene transcription. Small-molecule inhibitors of β-catenin or FAK synergize with vemurafenib BRAF inhibitor to prevent *BRAF* V600E colorectal cancer cell proliferation *in vitro* and xenograft tumor growth in mice. This study complements findings linking FAK to β-catenin in intestinal tumorigenesis, resistance to radiotherapy, and cancer stem cell survival. Thus, FAK activation may occur as a frequent tumor cell "adaptive resistance" mechanism. Although FAK (*PTK2*) is not mutated in most cancers, targeting FAK activity in combinational approaches may limit tumor cell escape mechanisms and enhance durable responses to treatment. *Mol Cancer Ther; 17(4); 719–23. ©2018 AACR.*

Colorectal cancer progression is a disease driven by the accumulation of genetic aberrations and oncogenic mutations. Colorectal cancer tumors harboring *BRAF* V600E mutations are generally treatment-resistant and have an extremely poor prognosis (1). *KRAS*, a predictive biomarker in the management of colorectal cancer, functions upstream of *BRAF* and mutations in these oncogenes activate the mitogen-activated protein kinase (MAPK/ERK) signaling cascade. Unlike melanoma, various BRAF inhibitors are ineffective as monotherapy in *BRAF*-mutated colorectal cancer (2). This may be due in part to the rapid activation of EGFR or phosphoinositide 3-kinase (PI3K) via proposed feedback activation loops or breakthrough signaling in response to pharmacological BRAF inhibition. Unfortunately, combinations of BRAF, EGFR, or PI3K inhibitors have limited efficacy in treating *BRAF* V600E colorectal cancer tumors (3). Thus, an alternative switch or BRAF "bypass" signaling pathway activation may continue to drive tumor growth upon BRAF inhibitor administration.

Focal Adhesion Kinase Activation in Response to BRAF Inhibition: Connections to Wnt/β-Catenin

In this issue, Chen and colleagues (4) show that increased focal adhesion kinase (FAK) tyrosine (Y) Y397 phosphorylation (a marker of FAK activation) occurs within 4 to 8 hours after BRAF inhibitor (vemurafenib, dabrafenib, GDC-0879, PLX7904, or LY3009120) treatment of colorectal cancer cells containing *BRAF* V600E mutations (Colo205, Colo741, HT-29, LS411N, SW1417, and WiDr) but not in colorectal cancer cells with wildtype *BRAF* (DLD-1, RKO, and SW48). Sustained FAK activation after vemurafenib addition (measured up to 48 hours) occurred in the presence of EGFR (erlotinib), MEK1 (trametinib), and ERK2 (SCH772984) inhibitors, suggesting that FAK activation was may be independent of BRAF–MEK–ERK–EGFR pathway reactivation. Notably, FAK Y397 phosphorylation was prevented by co-administration of FAK inhibitor (PF562271) and the combination of vemurafenib plus PF562271 potently blocked *BRAF*-mutant cell growth under conditions whereby ERK2 remained active. Together, these results suggest that FAK activation is distinct from a "BRAF pathway reactivation" process and that signaling linkages to targets other than ERK2 function to promote *BRAF* V600E-mutant cell growth (Fig. 1).

FAK is a cytoplasmic tyrosine kinase that is best characterized as mediating integrin receptor signals from matrix proteins controlling cell movement and survival (5). FAK expression and Y397 phosphorylation are elevated in several advanced-stage solid cancers (6) and it is believed that FAK signaling is compartmentalized through interactions with a variety of effector proteins that localize FAK either to focal adhesions, endocytic vesicles, cell–cell junctions, or the cell nucleus (5). Although Chen and colleagues (4) did not investigate where Y397-phosphorylated FAK was localized in vemurafenib-treated *BRAF* V600E-mutant colorectal cancer cells, Jing Hu's group previously showed that FAK promotes Wnt/β-catenin pathway activation and intestinal tumorigenesis by phosphorylating glycogen synthase kinase-3 beta (GSK3β) at Y216 (7).

GSK3β Y216 phosphorylation promotes the binding of ubiquitin E3 ligase β-TrCP (β-transducin repeats containing

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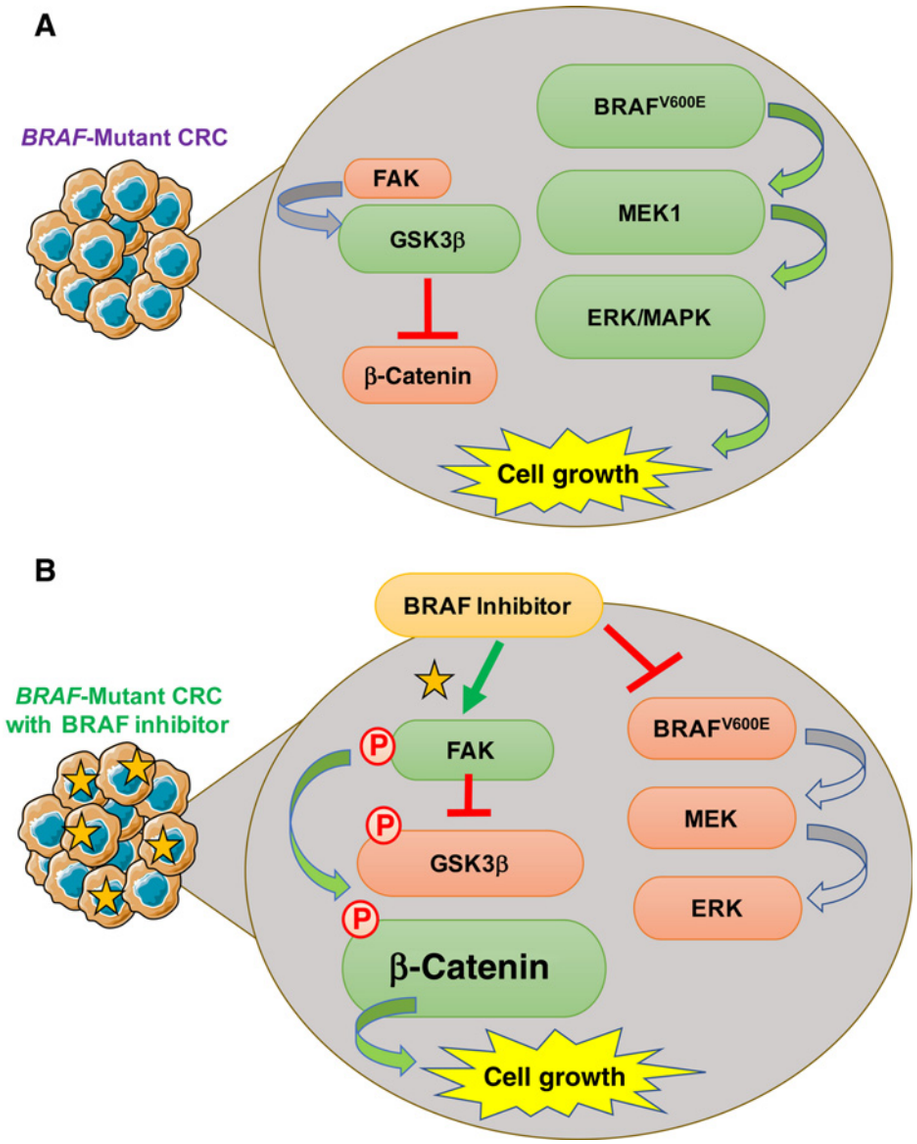

Figure 1.
Schematic of the BRAF/MEK/ERK pathway and an alternative bypass pathway in *BRAF*-mutant colorectal cancer. **A,** In the absence of BRAF inhibition, the BRAF/MEK/ERK pathway is active and supports cell growth. FAK is inactive, whereas GSK3β is activated and inhibits β-catenin. **B,** Upon BRAF inhibition, the BRAF/MEK/ERK pathway is inhibited. FAK is phosphorylated and inhibits GSK3β, allowing for bypass activation of β-catenin and continued cell proliferation.

protein) and subsequent ubiquitination and degradation of GSK3β. Because GSK3β activity is a negative regulator of β-catenin, FAK phosphorylation of GSK3β at Y216 indirectly results in enhanced β-catenin stabilization by lowering GSK3β levels. Notably, pharmacological inhibition of FAK activity (7) or conditional FAK knockout (8) suppressed intestinal tumorigenesis in mice heterozygous for tumor suppressor *adenomatous polyposis coli* (APC)—a major regulator of the β-catenin "destruction complex" with GSK3β, axin, and casein kinase 1. Conditional FAK knockout has also revealed a role in β-catenin-driven hepatocarcinogenesis (9) and FAK can enhance β-catenin tyrosine phosphorylation and activation in endothelial and breast carcinoma cells (10, 11). Together, these studies point to the importance of FAK in β-catenin regulation within multiple cell types.

In the current study by Chen and colleagues (4), treatment of HT-29 cells with BRAF inhibitors (dabrafenib, GDC-0879, or vemurafenib) resulted in a time-dependent increase in FAK Y397 phosphorylation, GSK3β Y216 phosphorylation, and β-catenin protein levels. Verurafenib increased nuclear β-catenin accumulation and Wnt target gene transcription in *BRAF*-mutant cells, which was independent of microsatellite instability or APC mutation status. FAK inhibitor treatment or knockdown of FAK mRNA levels prevented vemurafenib-induced β-catenin accumulation. Co-targeting the FAK/β-catenin pathway with either PF562271 or ICG-001 (a Wnt/β-catenin antagonist) in combination with vemurafenib, resulted in decreased cell proliferation *in vitro* and the synergistic prevention of HT-29 and *BRAF* V600E patient-derived xenograft tumor growth in mice. The results of Chen and colleagues (4) are notable for the identification of a novel FAK to Wnt/β-catenin parallel or "bypass" signaling mechanism "triggered" by BRAF inhibitor treatment of *BRAF* V600E-mutant colorectal cancer (Fig. 1).

A Multi–FAK-Torial Linkage to Adaptive Resistance

One of the open questions remaining is to determine the mechanism of how BRAF inhibitor addition triggers elevated FAK Y397 phosphorylation. Because PF562271 FAK inhibitor prevents vemurafenib-induced FAK Y397 phosphorylation, intrinsic FAK activity is needed. As increased FAK Y397 phosphorylation occurs in HT-29 tumors after vemurafenib treatment of mice, and after 4 to 24 hours treatment of HT-29, Colo205, Colo741, LS411N, and WiDr cell treatment with vemurafenib, LY3009120, PLX7904, or GDC-0879 BRAF inhibitors in cell culture, increased FAK Y397 phosphorylation likely involves a specific but indirect tumor-cell intrinsic mechanism. As this effect was not observed in colorectal cancer cells without BRAF mutation, the cells are likely primed for a "switch" to an adaptive resistance mechanism. However, the slow time frame makes it difficult to establish cause-effect relationships of potential changes in autocrine-paracrine factor production or the release of FAK inhibitory "restraint" through alterations in protein–protein interactions.

FAK activation is regulated by both conformational changes and specific recruitment to active sites of intracellular signaling (6). Although experimental results in Chen and colleagues (4) support the notion that FAK activation upon BRAF inhibitor treatment is independent of ERK/MAPK pathway reactivation, it is possible that constitutive ERK/MAPK pathway activation in *BRAF* V600E-expressing cells may also impact FAK in a negative regulatory manner. In particular, ERK/MAPK kinase-mediated phosphorylation of FAK at serine (S) S910 has been shown to result in FAK Y397 de-phosphorylation by tyrosine phosphatase action (12). Thus, reduction of constitutive ERK/MAPK activity *BRAF* V600E-expressing cells by BRAF inhibitor addition could possibly lead to increased FAK Y397 phosphorylation associated with the decrease in ERK/MAPK-mediated FAK S910 phosphorylation.

Notably, increased FAK Y397 phosphorylation is also part of an intrinsic adaptive resistance mechanism of melanoma cells to RAF inhibition (13) and involved in acquired sorafenib (a combined tyrosine kinase and BRAF inhibitor) resistance of *KRAS*-mutant lung adenocarcinoma cells (14). Moreover, tumor stromal responsive changes to BRAF inhibitor administration revealed an important role for matrix-integrin-FAK signaling in creating a protective tumor microenvironment (15). Importantly, pharmacological BRAF and FAK inhibition exhibit synergy in both *BRAF* V600E melanoma and colorectal cancer pre-clinical models.

How FAK/β-catenin pathway activation could be pre-wired for activation may be associated with the generation and survival of cancer stem cells (CSC). Both FAK and Wnt/β-catenin signaling have been connected to CSC plasticity and chemotherapy resistance (11). Chen and colleagues (4) detected increased expression of Wnt pathway targets SOX9 and LGR5 in vermurafenib-treated patient-derived xenograft tumors in mice, suggestive of an increase in a CSC pool. However, it remains unknown whether $LGR5^{+}$ colorectal cancer cells exhibit elevated FAK Y397 phosphorylation and nuclear β-catenin enrichment. Another potential connection between FAK/β-catenin and CSCs may also involve a Yes-associated protein 1 (YAP1) transcriptional complex. β-catenin forms a complex with YAP1 in the nucleus (16) and a FAK to YAP1 signaling axis supports stem cell-based tissue renewal in mice (17). Moreover, evidence is also accumulating that FAK and Wnt/β-catenin signaling are also supporting human ductal carcinoma *in situ* breast cancer (18) and *KRAS* mutated non–small cell lung cancer (19) resistance to radiotherapy. FAK inhibition resulted in susceptibility to ionizing radiation treatment associated with elevated DNA damage. Taken together, CSC-enriched populations exhibit resistance to radiotherapy and chemotherapy treatment—a similar population of cells that exhibit elevated FAK Y397 phosphorylation and Wnt/β-catenin activity.

Combinatorial Clinical Trial Testing of FAK Inhibitors

FAK inhibitor administration to mice exhibits antitumor activity as a monotherapy (6) and early-phase clinical trials with various FAK inhibitors have revealed an acceptable safety profile with 15% to 20% of unselected patients exhibiting disease stabilization (20, 21). In addition to the studies described above, support for combinatorial approaches using FAK inhibitors comes from pre-clinical studies showing that

Table 1. Early-phase clinical trials using FAK inhibitor as combination therapy

	Clinical trial ID	FAK inhibitor	Malignancy	Phase	Other agent(s)	Results (if any)
Completed trials	NCT01938443	GSK2256098	Advanced solid tumors	Ib	Trametinib (MEK inhibitor)	Stable disease >6 weeks = 67%[a]
	NCT01778803	VS-6063	Ovarian	I/Ib	Paclitaxel	Disease control rate = 64%[b] Objective response rate = 18%[c]
Ongoing trials	NCT02523014	GSK2256098	Meningioma	II	Vismodegib (hedgehog pathway inhibitor)	
	NCT02546531	VS-6063	Advanced solid tumors	I	Pembrolizumab (PD-1 inhibitor), gemcitabine	
	NCT02758587	VS-6063	Pancreatic, mesothelioma, non-small cell lung cancer (NSCLC)	I/IIA	Pembrolizumab (PD-1 inhibitor)	
	NCT02943317	VS-6063	Ovarian	I/II	Avelumab (PD-L1 inhibitor)	
	NCT03287271	VS-6063	Ovarian	I/II	Carboplatin, paclitaxel	

[a]Stable disease includes those who do not meet criteria for progression (at least 20% increase of target lesions), partial response (at least 30% shrinkage of target lesions), or complete response (disappearance of all target lesions).
[b]Disease control rate includes stable disease, partial responses, and complete responses.
[c]Objective response rate includes partial responses and complete responses.

tumor-intrinsic FAK signaling supports pancreatic and squamous cell carcinoma tumor immune evasion via modulation of cytokine expression and effects on tumor-infiltrating T cells (22, 23). Pharmacological FAK inhibition was associated with a depletion of T regulatory cells and an enhancement of $CD8^+$ T-cell–mediated antitumor response. Importantly, FAK inhibition rendered unresponsive *KRAS* and p53 tumor-suppressor mutant pancreatic tumors responsive to T-cell immunotherapy and PD-1 checkpoint inhibitor antagonists (23). Mouse survival was greatest using a combinatorial treatment of gemcitabine, FAK inhibitor, and anti–PD-1 antibody therapy. These studies are supporting phase I/II clinical trials evaluating FAK inhibitor combination with pembrolizumab (PD-1 inhibitor) in pancreatic cancer, mesothelioma, and non–small cell lung carcinoma (Table 1).

Other combinatorial approaches have targeted key cell signaling molecules involved in metastasis and cell proliferation. Related to the findings from Chen and colleagues (4), a phase Ib trial is evaluating the MEK inhibitor trametinib combined with GSK2256098 (a FAK inhibitor) in patients with advanced solid tumors (NCT01938443), with particular interest in mesothelioma, where synergy has been demonstrated with this drug combination *in vitro*. Vismodegib, an inhibitor of smoothened receptor (SMO, part of the hedgehog signaling pathway), with GSK2256098 is being tested in a phase II study of patients with meningiomas harboring mutations in SMO or PTCH1 (protein patched homolog 1), a related signaling protein (NCT02523014). By co-targeting the FAK/β-catenin pathway in addition to the mutated hedgehog pathway, there is potential to prevent or combat adaptive resistance.

Advanced ovarian cancer presents a unique treatment challenge because, although tumors are initially sensitive to platinum-based chemotherapy, patients recur and ultimately develop resistance to chemotherapy, with few agents demonstrating antitumor activity in this setting. Three trials of combination therapy with FAK inhibitor specifically target recurrent ovarian cancer and aim to overcome mechanisms of drug resistance. In a phase I/Ib trial of 22 patients with recurrent ovarian cancer, the FAK inhibitor VS-6063 was administered in combination with paclitaxel, resulting in a 64% disease control rate, including two complete and two partial responses (NCT01778803). The Re-sensitization of carboplatin-resistant Ovarian Cancer with Kinase Inhibition of FAK (ROCK-IF) trial is a phase I/II study of VS-6063 with paclitaxel and carboplatin in patients with recurrent or persistent platinum-resistant tumors (NCT03287271). Inhibition of the FAK/β-catenin pathway may restore sensitivity to carboplatin, thus enabling a treatment option for platinum-resistant ovarian cancer.

Conclusion

The search for novel treatment approaches in drug-resistant cancers remains a focus of inquiry in the preclinical and clinical arenas. Activation of a FAK signaling linkage to β-catenin supports tumor cell survival in the setting of cytotoxic or targeted therapy. Targeting FAK in combination with therapies directed at other pathways may be a promising approach to turn off the adaptive "switch" promoting tumor cell proliferation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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