Editorial

From tumorigenesis to microenvironment and immunoregulation: the many faces of focal adhesion kinase and challenges associated with targeting this elusive protein

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Focal adhesion kinase (FAK) has long been confirmed to have a major role in cancer as it involved in virtually every aspect of tumorigenesis and tumor progression (1,2). Known for being significantly overexpressed in over 80% of solid tumors, and even hematological cancers (3,4), FAK drives tumorigenesis by regulating many of the traditional hallmarks of cancer (5,6). Research continues to demonstrate FAK's involvement in multiple aspects of cancer, primarily due to the role of FAK as an oncogenic scaffold (7). FAK has docking sites at both the N- and C-termini of the protein, allowing these regions to interact with a multitude of binding partners driving proliferation, survival, invasion/migration, and angiogenesis. As FAK expression is minimal in surrounding normal tissue, these phenotypes are a defining characteristic of FAK overexpression in cancer. Additionally, FAK plays a role in the sequestration of pro-apoptotic proteins (8), the manipulation of the tumor microenvironment (TME) (9), the immune response (10), and anoikis (11), all of which demonstrate the importance of FAK as a continually evolving, multifaceted protein in tumor biology.

Despite FAK's ability to drive malignant phenotypes, identifying FAK as a novel therapeutic target and developing effective therapeutics that target FAK have been difficult and elusive. Why has it been so hard to drug FAK? FAK does not have a specific mutation that can be targeted like BRAF (V600E) (12) nor is it a receptor like HER2 (13) or EGFR (14). Compared to oncogenes that require a mutation to trigger a malignant phenotype, the overexpression of wild-type FAK in tumors relative to the basal expression in surrounding normal tissue is sufficient to drive malignancy within the tumor and TME (15,16).

The primary driver of FAK function is through the phosphorylation of Y397 which allows for the subsequent activation of downstream tyrosines 861 and 925 by Src. This Y397 autophosphorylation site is critical for development of FAK-targeted therapeutics, by developing small molecule inhibitors specific for the ATP-binding site of the FAK-kinase domain to inhibit FAK-kinase function and autophosphorylation of Y397. To date, efforts to drug FAK based exclusively on its kinase function have not shown dramatic clinical responses and are made more challenging by the lack of an appropriate biomarker of response. For example, there are recent failed clinical trials of defactinib, including a MERLIN biomarker-based clinical trial that was terminated (NCT01870609) (17), raising the question of whether strict FAK kinase inhibitors are effective to target FAK in patients. Central dogma surrounding FAK inhibition has focused on inhibition of the FAK kinase enzyme only, which has led to a lag in the development of therapeutics targeting the non-kinase function of FAK (18,19).

It becomes a challenge to identify the best strategy to target a protein like FAK. It has been clearly established

that Y397 phosphorylation is the critical mediator of FAK function, allowing for downstream activation of FAK's own kinase domain and subsequent effector tyrosines 861 and 925 to be phosphorylated by Src. All three of these activated phosphotyrosines function as new scaffolding hubs that maintain proliferation, survival, migration/invasion, and angiogenesis. A critical factor in effectively targeting FAK is to shut off Y397 signaling but, unfortunately, FAK kinase inhibitors do not effectively do this. Creating kinase domain inhibitors is a simpler method than attempting to identify FAK-scaffold inhibitors. However, there is now enough evidence to support the cross-reactivity and limited therapeutic efficacy of these kinase inhibiting small molecules. Nonetheless, these tyrosines are additional scaffold sites that, in theory, can be targeted with the development of protein-protein interaction (PPIs) inhibitors designed to target the binding interfaces between two proteins (20).

To overcome this challenge of targeting FAK effectively, we must look to the creation of small molecule inhibitors that can block the critical signaling events on the scaffold. The creation of FAK-scaffold inhibitors may have seemed unachievable years ago, but now with advances in *in silico* rational based drug design we can accurately target the multiple and unique binding interfaces of FAK. Ideally, we want to prevent the multitude of functions that each domain of FAK demonstrates. By targeting the FERM domain we can inhibit interactions with receptor tyrosine kinases (RTKs), which we know signal indirectly and now, directly, with this domain (21). The FERM domain is not only a hub for RTKs but also intracellular survival signals, such as p53, MDM2, and RIP. Additionally, Y397 resides within a flexible linker region that joins the FERM domain to the central kinase domain that is also accessible by RTKs and quite possibly additional intracellular TKs, i.e., Src.

Recently our group has shown that RTKS protect Y397 by maintaining its phosphorylation. These RTKs can directly phosphorylate FAK at Y397 independent of FAK-kinase function (i.e., FAK-kinase inhibitor defactinib, stable overexpression of kinase-dead FAK in $FAK^{-/-}$ MEFs) through the direct interaction of FAK and RTKs (21). This novel observation challenges the idea that FAK activation of Y397 is a mechanism regulated by FAK autophosphorylation and not by outside kinases. This demonstrates the importance of Y397 activation, suggesting the need for novel drug development approaches such as PPIs designed to these newly discovered binding interfaces (22) as FAK remains a lynchpin and key signaling transducer.

The C-terminal region of FAK, which includes the FAT domain, is another appropriate for the development of targeted PPIs. The FAT domain is functionally relevant for proper localization of the FAK molecule to focal adhesion complexes, whose structural dynamics regulate focal adhesion turnover, and in turn, drive migration, invasion, and angiogenesis. Additionally, Y925, which resides on the FAT domain, is the key regulator of focal adhesion turnover and is a juncture for paxillin and GRB2 binding dynamics. Blocking Y925 phosphorylation opens up a new realm of FAK inhibition, not by blocking a catalytic domain but by allosterically inhibiting the binding interfaces and tyrosines responsible for regulating focal adhesion turnover and FAT domain behaviors (23).

As we gain more insight into why FAK is expressed at such high levels in malignant cells, we are learning how FAK manipulates the immune landscape of the tumor, i.e., TME, platelets, immune response, T cell receptor signaling, etc. This includes the induction of FAK signaling in the tumor stroma in BRAF-mutant melanoma (15), the regulation of Tregs and $CD8^{+}$ T cells through nuclear FAK activation of chemo/cytokines (10), and, most recently, FAK expression in platelets inducing tumor growth after anti-angiogenic therapy withdrawal (9). This novel finding suggests that systemic FAK inhibition to the TME in combination with anti-angiogenic therapy, such as bevacizumab or pazopanib may inhibit tumor regrowth by reducing platelet migration to the tumor. Beautifully demonstrated through the use of the platelet-specific knock-out, FAK is once again shown to be a modulator of the pro-cancer phenotype this time through the microenvironment (9). The resurgence of angiogenesis after anti-angiogenic drugs have been stopped appears to be overcome by the inhibition of FAK. As one of the main functions of FAK is regulating cell adhesion, it is no surprise that inhibiting FAK in platelets (24) would inhibit tumor revascularization by preventing platelet attachment. Haemmerle reiterates FAK's role in the tumor; demonstrating FAK as a manipulator of the TME by regulating platelet activity.

Haemmerle's data aligns with the literature in that the complete knockdown of FAK was better to inhibit FAK-mediated phenotypes in the tumor, even more than FAK-kinase inhibitors alone. This is another example of how the total inhibition of FAK function is the best approach to targeting FAK in cancer. This allows for the creation of multifaceted FAK inhibitors, ranging from traditional FAK-kinase inhibitors to novel FAK-scaffold inhibitors. Due to

the complexity of FAK signaling, there is a need to develop a more effective link between FAK's complex role in cancer biology with the development of targeted therapeutics against FAK. As scaffold inhibitors are in their infancy (25) it becomes an underlying concern that we continue to rely on only kinase inhibitors that can lead to tumor resistance. The simultaneous targeting of both scaffolding domains of FAK not only has the potential to circumvent kinase domain related complications but also to disrupt scaffolding interactions and subsequent downstream signaling, lending additional efficacy to the overall approach. Additionally, inhibition of the FAK scaffold (non-kinase) domains may prove similarly effective to inhibit FAK function in the TME. With the rapidly increasing use of immune checkpoint inhibitors (CPI) and other immuno-oncology (I-O) therapeutics in tandem with our knowledge of FAK in manipulating the immune landscape and TME in cancer, moving in a direction of incorporating FAK-targeted inhibitors with I-O therapies seems plausible. Given the multitude of processes and signaling pathways that require FAK, it would be naïve to think that one single FAK inhibitor would be effective. In the era of precision targeted therapeutics, it is more likely that FAK inhibitors would need to be tailored to an individual patient's tumor utilizing more effective therapeutics that extend beyond the FAK-kinase domain.

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