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The focal adhesion kinase inhibitor PF-562,271 impairs primary CD4+ T cell activation☆

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ABSTRACT

The focal adhesion kinase inhibitor, PF-562,271, is currently in clinical development for cancer, however it is not known how PF-562,271 affects T cell function. Here, we demonstrate inhibitory effects of PF-562,271 on the activation of primary human and mouse T cells. PF-562,271 inhibits T cell receptor signaling-induced T cell adhesion to intercellular adhesion molecule-1 and T cell interactions with antigen-presenting cells. An additional focal adhesion kinase inhibitor, PF-573,228, and genetic depletion of focal adhesion kinase also impair T cell conjugation with antigen-presenting cells. PF-562,271 blocks phosphorylation of the signaling molecules zeta chain associate protein of 70 kDa, linker of activated T cells, and extracellular signal-regulated kinase, and impairs T cell proliferation. The effects observed on T cell proliferation cannot solely be attributed to focal adhesion kinase inhibition, as genetic depletion did not alter proliferation. The effect of PF-562,271 on T cell proliferation is not rescued when proximal T cell receptor signaling is bypassed by stimulation with phorbol-12-myristate-13-acetate and ionomycin. Taken together, our findings demonstrate that focal adhesion kinase regulates integrin-mediated T cell adhesion following T cell receptor activation. Moreover, our findings suggest that PF-562,271 may have immunomodulatory effects that could impact its therapeutic applications.

1. Introduction

Focal adhesion kinase (FAK) plays a central role in cancer cell adhesion, migration, and cell cycle progression and represents an intriguing anti-cancer target (reviewed in [1]). Previous studies have demonstrated that FAK expression is amplified in human tumors, including breast cancer, and mammary specific deletion of FAK impairs tumor progression and metastasis in mouse models [2,3]. Therefore, there has been substantial interest in developing agents that block FAK signaling. At least two FAK inhibitors are currently in clinical trials for treatment of pancreatic, head and neck, and prostate cancers, including PF-562,271 [4]. PF-562,271 (Fig. 1A) is an inhibitor of FAK and Pyk2, with a 4-fold increase in sensitivity for FAK over Pyk2 in cultured cells [5]. Animal studies with this compound show dose-dependent decreases in tumor volume for prostate, breast, pancreatic, colon, glioblastoma, lung, bone and hepatic tumors [5–8]. This compound has reached Phase II clinical trials and has shown promising effects for several tumor types with few adverse effects [9,10]. However, it is not known whether or not FAK inhibitors such as PF-562,271 affect T cell function.

T cells express both FAK and its homolog Pyk2, which are tyrosine phosphorylated in response to chemokines [11], signaling from integrins (including lymphocyte function-associated antigen 1 (LFA-1, αLβ2) [12], and ligation of the T cell receptor (TCR)) [13,14]. Pyk2 knockout mice [15] are viable and fertile and demonstrate impaired macrophage [16] and CD8+ T cell function [17]. CD8+ T cells from Pyk2-deficient mice demonstrate a defect in LFA-1 mediated adhesion to intercellular adhesion molecule 1 (ICAM-1) and a decrease in effector but not memory T cell responses [17]. There is evidence that at least some patients with Systematic Lupus Erythematosus have increased Pyk2 expression and phosphorylation in circulating peripheral blood mononuclear cells [18]. However, because FAK knockout mice are not viable, studies on the functions of FAK in primary T cells have lagged those of its homolog.

☆ HEB has recently become affiliated with Pfizer. The experiments on PF-562,271 were performed prior to this affiliation and were performed solely at the University of Wisconsin-Madison by the other authors, who have no affiliation with Pfizer.

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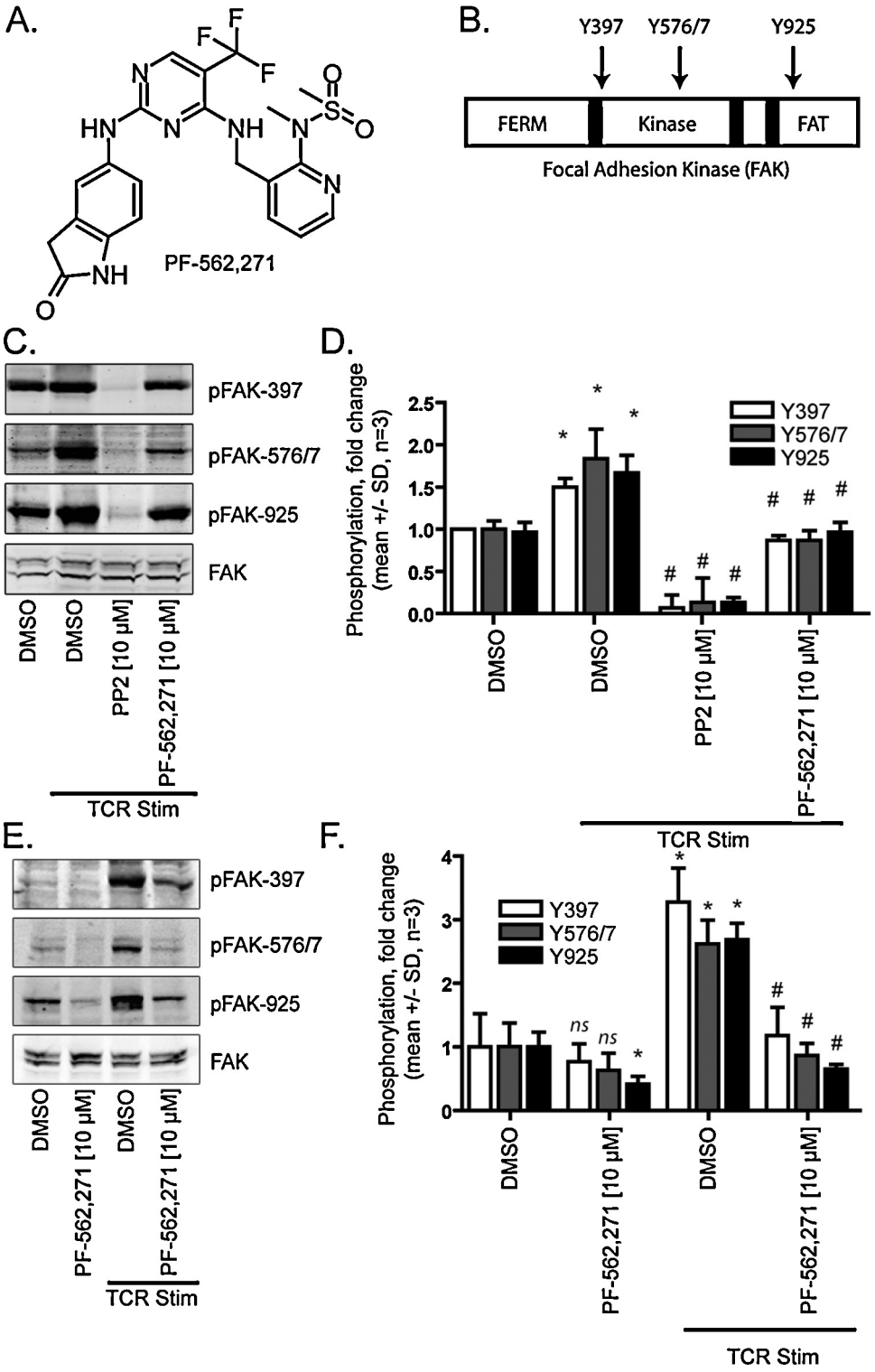

Fig. 1. PF-562,271 inhibits site-specific phosphorylation of FAK in human peripheral blood T cells. (A) Chemical structure of the FAK inhibitor PF-562,271. (B) Domain architecture of FAK (FERM = 4.1 protein, ezrin, radixin and moesin, FAT = focal adhesion targeting). (C) Western Blot analysis of FAK phosphorylation relative to total FAK in the presence or absence of 10 μM test compounds. Blots are representative of three independent experiments. (D) Quantification of FAK phosphorylation (mean ± SD, $n = 3$, $p < 0.05$ relative to DMSO control (*) or relative to TCR control (#), ANOVA). (E) Western Blot analysis of FAK phosphorylation relative to total FAK in the presence or absence of 10 μM PF-562,271. Blots are representative of three independent experiments. (F) Quantification of FAK phosphorylation (mean ± SD, $n = 3$, $p < 0.05$ relative to DMSO control (*) or relative to TCR control (#), ANOVA) ns = not significant.