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**FAK goes nuclear to control anti-tumor immunity – a new target in cancer immuno-therapy.**

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## **Abstract**

Evading the anti-tumor immune response is important for the survival and progression of cancer. Recently, we identified an unexpected role for nuclear FAK activity in the control of tumor Treg levels and immune evasion by regulating chemokine and cytokine transcription in cancer cells. We proposed a potentially new purpose for FAK kinase inhibitors, which can cause immune-mediated tumor regression.

## **Commentary**

Unleashing the anti-tumor immune response is proving to be a very exciting therapeutic option in the fight against cancer. Recently, we reported a new and surprising function for Focal Adhesion Kinase (FAK) in regulating the immunosuppressive tumor environment <sup>1</sup>. This led us to propose repurposing FAK inhibitors as a new class of immuno-modulatory therapy.

Briefly, we noticed that the ability of FAK-deficient tumor cells (in our case Squamous Cell Carcinoma (SCC)s from which *fak* had been deleted) to grow was quite different in mice that had a functional adaptive immune system (the syngeneic FVB host) when compared to mice that did not (CD1 nude mice). It transpired that nuclear FAK activity drives selective chemokine and cytokine transcription, resulting in dysfunction of cytotoxic CD8 T-cells and elevated intra-tumoral Tregs – together causing suppression of the CD8 T-cell anti-tumor immune response (**Figure 1**). Lying at the heart of this immuno-modulatory activity was a FAK-regulated chemokine ligand-receptor paracrine signaling axis between SCC cancer cells and tumor infiltrating Tregs. What we uncovered was a function for FAK in controlling tumor evasion via induction of an immuno-suppressive tumor microenvironment.

These findings were surprising for a number of reasons:

**Firstly**, when FAK is examined by microscopy, it appears to localize predominantly to integrin complexes at sites of cell adhesion to extracellular matrix – the so-called focal adhesions (FAs). As such, we assumed its role in cancer was most likely biological processes controlled by intracellular signaling from FAs, as have been documented over the years, eg. adhesion, polarization, migration, invasion and survival <sup>2</sup>. FAK is upregulated in many cancers <sup>2</sup>, and small molecule inhibitors from several pharmaceutical companies are in clinical development <sup>3,4</sup>, with some showing clinical activity as a single agent <sup>5</sup>. What our work suggests is that good responses to FAK inhibitors may be via effects on the immune system, possibly in addition to the more widely predicted tumor cell autonomous effects on survival, proliferation and invasion.

**Secondly**, we had not considered that the most profound effects of FAK in cancer might come from its activities in the nucleus. In fibroblasts, endothelial cells, and muscle cells, low steady-state levels of nuclear FAK have been identified, with increased nuclear accumulation occurring in response to cellular stress <sup>6</sup>. We found no evidence of nuclear FAK in normal skin keratinocytes (although we did not subject these to stress), implying that nuclear accumulation of FAK in malignant keratinocyte SCCs was associated with the cancerous phenotype. This raises the exciting possibility that the immuno-regulatory function of nuclear FAK may be ‘specific’ to cancer, providing a window of therapeutic opportunity. While the full extent of FAK’s nuclear functions remain to be elucidated, we note that activated (phosphorylated on Tyr-397) nuclear FAK has been identified as a prognostic indicator of poor clinical outcome in colorectal cancer <sup>7</sup>.

**Finally**, having discovered that nuclear FAK was driving an immuno-suppressive environment, we were further surprised to find that FAK was associated with

chromatin, and in wider proteomic and contextualized network analysis (using Ccl5 as the exemplar FAK-regulated promoter), that FAK binds to components of the basal transcription machinery and upstream regulators of key sequence-specific transcription factors. While the mechanisms that govern specificity require to be worked out, we conclude that FAK scaffolds, in a kinase dependent manner, selective regulators of chemokine transcription, in turn leading to Treg recruitment and immune evasion.

Our findings raise a number of interesting corollaries: Since FAK inhibitors are broadly well tolerated, and we do not know of any autoimmune side effects, these may have direct therapeutic benefit with relatively minimal side effects when the tumor cells are immunogenic. Using a FAK inhibitor currently in clinical development (clinicaltrials.gov NCT01849744), VS-4718 <sup>4</sup>, we observed immune-mediated SCC tumor regression (effectively curing these mice), and this was associated with decreased intra-tumoral Tregs and an elevated anti-tumor CD8 T-cell response.

Of course, not all tumors are highly immunogenic, and tumor-associated cytotoxic T cells typically have an exhausted phenotype – features that need to be overcome in therapeutic approaches. In pre-clinical murine cancer models, targeting components of the immuno-suppressive microenvironment including Tregs (and macrophages) has successfully released the brake on anti-tumor immunity, either alone or in combination with agents that stimulate T-cell activity <sup>8,9</sup>. In man, targeting the tumor immune environment has changed the treatment of melanoma; indeed, a clinical study combining agents that target Programmed Death Receptor-1 (PD-1) and Cytotoxic-T-Lymphocyte-associated Antigen-4 (CTLA-4), reported an impressive 53% of patients with an objective response resulting in greater than 80% reduction in tumor burden <sup>10</sup>.

Whilst there is ‘wind in the sails’ of immunotherapy as a potent arsenal in the fight against cancer, there is also the realization that modulating immune cell populations can result in severe autoimmune side effects, likely due to disrupting important homeostatic functions. For example, the impressive response rate of the combination of nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) in melanoma was accompanied by substantial side effects (clinical grade 3-4) in greater than 50% of patients <sup>10</sup>. Therefore, while significant progress has been made, and is undoubtedly hugely exciting, we need to find alternative effective combinations with improved tolerability. We propose that FAK inhibitors may represent one new class of better-tolerated immuno-modulatory agents. These could complement existing immunotherapy treatments, such as checkpoint blockade inhibitors, and clinical studies testing this hypothesis are planned.

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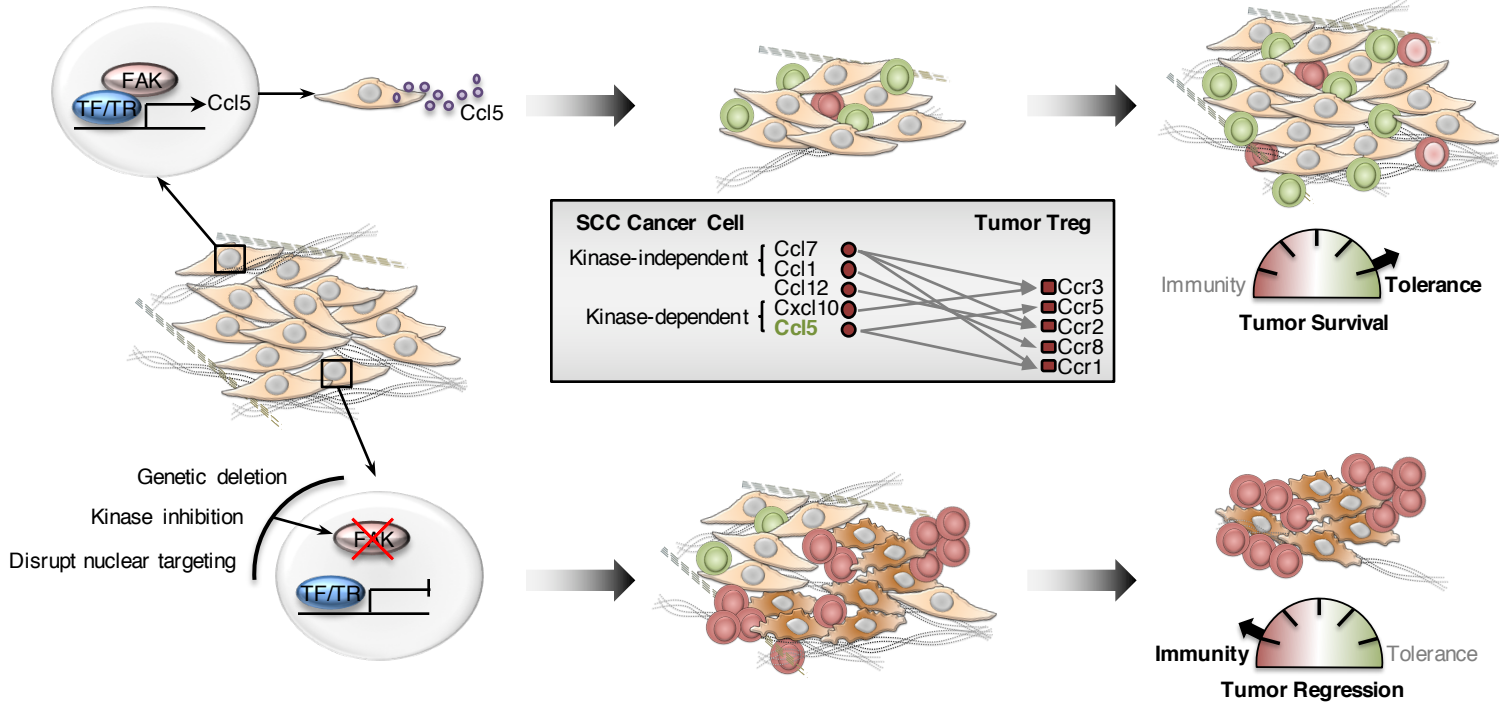
**Figure legend:**

**Figure 1. Schematic summary of our current understanding of how FAK signaling in SCC cells contributes to evasion of the anti-tumor immune response.**

1. Nuclear FAK drives chemokine / cytokine expression potentially through its interaction with transcription factors (TF) and transcriptional regulators (TR).

2. A chemokine ligand / receptor paracrine signaling axis between FAK-expressing cancer cells and tumor infiltrating Tregs drives elevated Treg levels in SCC tumors.

3. Elevated intra-tumoral Treg levels alter the CD8 T-cell / Treg ratio in favor of tumor tolerance, allowing SCC tumors to survive and grow.



4. Disrupting FAK through genetic deletion, kinase inhibition, or perturbing nuclear translocation results in loss of Ccl5 and TGFβ2 expression.

5. Perturbing FAK signaling alters the CD8 T-cell / Treg ratio in favor of anti-tumor immunity, resulting in CD8 T-cell-dependent tumor regression and improved prognosis.

