FAK Inhibition Re-sensitizes Platinum-resistant Serous Ovarian Cancer

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Abstract

Focal adhesion kinase (FAK), an intracellular tyrosine kinase, has been linked to mesothelial and breast cancer stem cell (CSC) survival. Here, we find that FAK activation is elevated in platinum (CP)-resistant ovarian cancer cells and that FAK tyrosine phosphorylation is increased after CP treatment of CP-sensitive ovarian cancer cells. Nanomolar levels of FAK inhibitor (VS-4718) selectively blocked CP-resistant ovarian carcinoma colony growth. Oral VS-4718 administration to mice reduced CP-resistant orthotopic tumor burden with a concomitant decrease in tumor-associated aldehyde dehydrogenase (ALDH) activity, a marker of ovarian cancer stem cells (CSC). Here, we find that FAK activation is elevated in platinum (CP)-resistant ovarian cancer cells and that FAK tyrosine phosphorylation is increased after CP treatment of CP-sensitive ovarian cancer cells. Nanomolar levels of FAK inhibitor (VS-4718) selectively blocked CP-resistant ovarian carcinoma methylcellulose colony growth. Oral VS-4718 administration to mice reduced CP-resistant orthotopic tumor burden with a concomitant decrease in tumor-associated aldehyde dehydrogenase (ALDH) activity, a marker of ovarian cancer stem cells (CSC). CRISPR-mediated FAK knockout or VS-4718 treated ovarian carcinoma cells exhibit diminished Oct-4 transcription factor and ALDH-1A1 CSC-associated protein marker expression. Co-administration of VS-4718 with CP-taxol chemotherapy reduced CP-resistant ovarian carcinoma spheroid growth. As CP activates FAK and that FAK inhibitors in combination with CP prevent recurrent and chemotherapy-resistant ovarian cancer.

FAK is activated (Y937 phosphorylation) by multiple cell receptors

Elevated FAK expression is a poor prognostic marker for ovarian cancer patients

Fig. 1. Elevated FAK gene (FAK) expression is a poor prognostic marker for ovarian cancer patients. (A) FAK gene amplification (TOSA) in ovarian cancer patients. (B) A large proportion of high FAK mRNA levels were observed in ovarian cancer patients with high FAK gene amplification. The Cancer Genome Atlas (TCGA) data for ovarian cancer was analyzed to assess the relationship between FAK mRNA levels and overall patient survival. The Kaplan-Meier estimator was used to evaluate overall survival for TCGA ovarian cancer patients with high FAK gene amplification. The differences in survival between TCGA ovarian cancer patients with high FAK mRNA levels and overall survival were statistically significant (log-rank test, **p<0.01). (Adapted from Sulzmaier et al., Nature Cancer Medicine 14: 536, 2014)

FAK is activated by cisplatin treatment

Fig. 2. Increased FAK Y397 phosphorylation after cisplatin treatment and in cisplatin-resistant ovarian cancer cells. A) Western blot analysis of pY397 FAK and total FAK in A2780 and OVCAR10 cells treated with cisplatin (10 µM) for the indicated times prior to cell lysis and immunoblotting by LICOR. B) Table: FAK activation and determination of IC50 values for A2780 and OVCAR10 cells treated with cisplatin at multiple concentrations. C) Representative image of methylcellulose colony growth of A2780 and OVCAR10 cells treated with cisplatin at multiple concentrations. D) CRISPR-mediated FAK knockout or VS-4718 treated ovarian carcinoma cells exhibit diminished Oct-4 transcription factor and ALDH-1A1 CSC-associated protein marker expression. Co-administration of VS-4718 with CP-taxol chemotherapy reduced CP-resistant ovarian carcinoma spheroid growth. As CP activates FAK and that FAK inhibitors in combination with CP prevent recurrent and chemotherapy-resistant ovarian cancer.

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Fig. 3. VS-4718 treatment reduces secondary ovarian tumor initiation frequency. (A) Parental OVCAR10 and A2780 cell growth in suspension is resistant to VS-4718. (B) VS-4718 prevents A2780-CP70 but not A2780 growth as spheroids. (C) Knockout of FAK disrupts Oct-4 transcription factor and ALDH-1A1 CSC-associated protein marker expression. Co-administration of VS-4718 with CP-taxol chemotherapy reduced CP-resistant ovarian carcinoma spheroid growth. As CP activates FAK and that FAK inhibitors in combination with CP prevent recurrent and chemotherapy-resistant ovarian cancer.

Mouse tumor model testing of cisplatin-plus/plus VS-4718 FAK Inhibitor Therapy

Fig. 4. VS-4718 FAK inhibition re-sensitizes platinum-resistant chemotherapy on cisplatin-resistant tumors. (A) Immunohistochemical analysis of methylcellulose colonies of A2780-CP70 cells treated with cisplatin (10 µg/ml), VS-4718 (5 µM), and cisplatin plus VS-4718. (B) Quantitation of methylcellulose spheroid colonies in DMSO or 0.1 µM VS-4718. Values are means ± SEM of 3 independent repeats (*** p < 0.001). (C) Western blot analysis of pY397 FAK and total FAK in A2780-CP70 cells treated with cisplatin (10 µM) for the indicated times prior to cell lysis and immunoblotting by LICOR. (D) Mean number of peritoneal ascites tumors in each group. The differences in peritoneal ascites tumors between VS-4718 treated groups and control groups were statistically significant (log-rank test, **p<0.01). (E) Non-parametric survival curve was used to analyze survival of each group. Data points represent mean values ± SD from individual mice. Bars are means ± SEM (**p<0.01). (B) VS-4718 administered to mice re-sensitized ovarian carcinoma cells to cisplatin and exhibited additive inhibitory effects on ovarian carcinoma spheroid growth. As CP activates FAK and that FAK inhibitors in combination with CP prevent recurrent and chemotherapy-resistant ovarian cancer.

Future Directions

FAK is activated by cisplatin treatment and elevated basal Y937 FAK phosphorylation occurs as a function of acquired cisplatin resistance. VS-4718 sensitizes cisplatin-resistant cells to platinum chemotherapy in vitro and in vivo. VS-4718 diminishes cisplatin-resistant cancer stem-like cell properties. FAK inhibitor combination with carboplatin-taxol chemotherapy may enable a more durable clinical response.

Image 1: Elevated FAK expression is a poor prognostic marker for ovarian cancer patients. (A) FAK gene amplification (TOSA) in ovarian cancer patients. (B) A large proportion of high FAK mRNA levels were observed in ovarian cancer patients with high FAK gene amplification. The Cancer Genome Atlas (TCGA) data for ovarian cancer was analyzed to assess the relationship between FAK mRNA levels and overall patient survival. The Kaplan-Meier estimator was used to evaluate overall survival for TCGA ovarian cancer patients with high FAK gene amplification. The differences in survival between TCGA ovarian cancer patients with high FAK mRNA levels and overall survival were statistically significant (log-rank test, **p<0.01). (Adapted from Sulzmaier et al., Nature Cancer Medicine 14: 536, 2014)

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