#6412 Development of a High-Throughput Assay Platform for the Screening of Selective Integrin Inhibitors in Cancer

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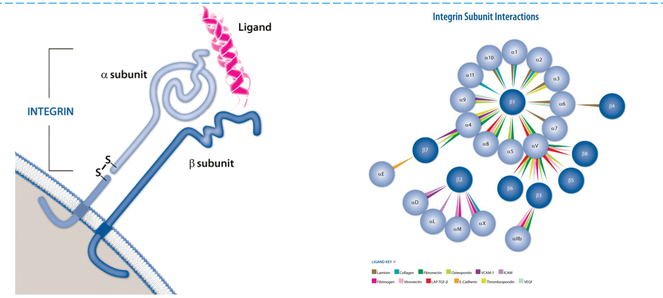
Abstract

Integrins are critical mediators of cancer progression, facilitating cancer cell invasion, survival in circulation, and metastatic outgrowth. Integrins result in cancer progression by activating pro-survival signaling pathways and remodeling the cancer microenvironment, and thus represent promising targets for anticancer treatments. To accelerate the discovery of novel integrin-targeted therapeutics, we developed an integrated platform of high-throughput screening assays for the evaluating integrin inhibitors of diverse modalities, including small molecules, peptides, antibodies, and antibody-drug conjugates. In this study, we developed a panel of cell-free fluorescence polarization (FP) assays in 384-well plate format using a Cy3B-RGD probe to screen compounds against multiple RGD-binding integrins (αvβ1, αvβ3, αvβ5, αvβ6, αvβ8 and α8β1). In addition, we generated HEK293 cell lines stably overexpressing integrins such as α8β1, and developed ELISA as well as IncuCyte based α8β1-Mfge8 competition binding assays for compounds screening in cell-based assays. The assay results were further validated using cancer cell lines endogenously expressing high levels of integrins such as α8β1, confirming compound binding and activity in a more physiologically relevant model.

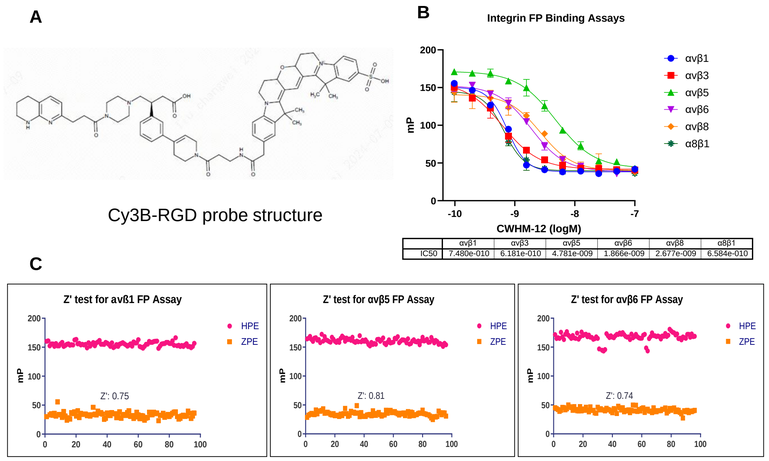
Introduction



- Integrins are transmembrane receptors that facilitate cell-extracellular matrix adhesion.
- Upon ligand binding, integrins activate signal transduction pathways that mediate cellular signals such as regulation of the cell cycle, organization of the intracellular cytoskeleton, and movement of new receptors to the cell membrane.
- Integrins are heterodimers, with two subunits: α (alpha) and β (beta). Integrins in mammals have twenty-four α and nine β subunits.

Results

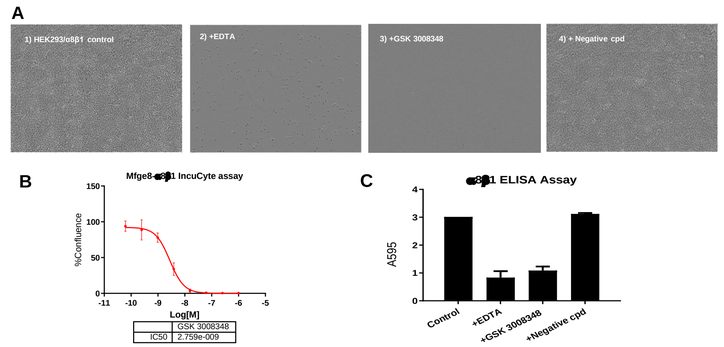
High-Throughput Biochemical Assays for Integrins



	αvβ1	αvβ3	αvβ5	αvβ6	αvβ8	α8β1
IC50	7.480e-010	6.181e-010	4.781e-009	1.866e-009	2.677e-009	6.584e-010

Figure 1. Cell-free fluorescence polarization (FP) assays. (A) Cy3B-RGD probe used in the FP assays. (B) Reference compound data of Integrin FP binding assays. (C) QC data for the Integrin FP binding assays.

Cell-based Assays for Integrins using Stable Cell Lines



	GSK 3008348
IC50	2.759e-009

Figure 2. Integrin α8β1 IncuCyte and ELISA assays using stable cell line. (A) Image data of integrin α8β1 IncuCyte assay. (B) Quantitative IncuCyte assay data with reference compound dose responses. (C) Integrin α8β1 EILSA assay data using HEK293/α8β1 stable cell line.

Results

Cell-based Assays for Integrins using Cancer Cells

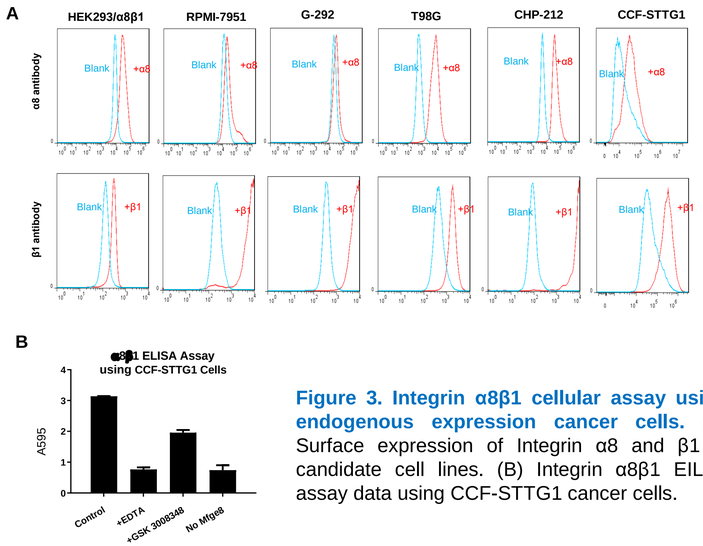


Figure 3. Integrin α8β1 cellular assay using endogenous expression cancer cells. (A) Surface expression of Integrin α8 and β1 in candidate cell lines. (B) Integrin α8β1 EILSA assay data using CCF-STTG1 cancer cells.

Summary

In summary, we have created a robust high-throughput screening platform that combines cell-free and cell-based assays. This integrated integrin assay platform enables the efficient evaluation of selective integrin inhibitors, accelerating the development of novel integrin inhibitors for target-based cancer treatment.

References

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