Integrin α6β4 Transcriptionally Upregulates Tryptophan Metabolism in Triple Negative Breast Cancer

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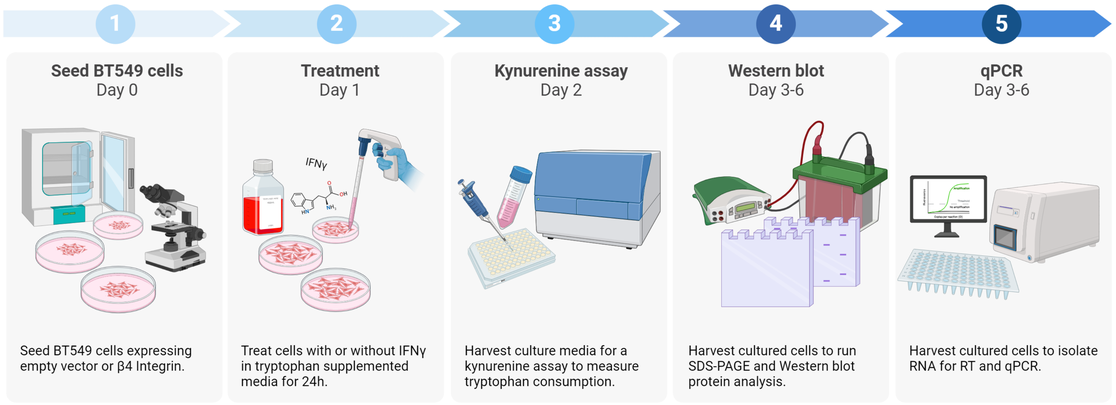
Abstract

Metabolic reprogramming is a hallmark of triple-negative breast cancer (TNBC). Tryptophan (Trp) metabolism in TNBC is upregulated through the kynurenine (Kyn) pathway, which produces immunosuppressive metabolites in the tumor microenvironment (TME) and is associated with immune evasion. Moreover, the integrin α6β4, which is reported to stimulate immune evasion, is overexpressed in TNBC and is responsible for nucleating cell-matrix adhesions and contributing to tumor survival, proliferation, angiogenesis, and metastasis. These functions of integrin α6β4 are mediated, in part, through signaling pathways or epigenetic regulation. While the significance of α6β4 in TNBC pathology is well established, its involvement in regulating important metabolic pathways has yet to be explored. Our study investigated the role of α6β4 in Trp metabolism using BT549 cells expressing either an empty vector (EV) or integrin β4, which results in the functional expression of integrin α6β4 on the cell surface. Whole genome bisulfite sequencing and RNA sequencing showed increased gene body methylation and promoter demethylation of the Trp-degradation enzyme indolamine 2,3 dioxygenase 1 (IDO1) gene and increased IDO1 expression in integrin α6β4-expressing cells. Additionally, we found that IDO1 expression strongly correlated with interferon-gamma (IFNγ) expression, a cytokine abundant in the tumor microenvironment, and that integrin α6β4 enhanced the IFNγ response signature. Using BT549 EV and β4 cells treated with or without IFNγ in Trp-supplemented media, we report that β4 expressing cells treated with IFNγ exhibit greater Kyn production than EV cells, which was determined by a fluorescence-based kynurenine quantification assay. Furthermore, western blot and qPCR analysis showed substantially increased IDO1 expression in BT549 β4 cells treated with IFNγ than in EV cells. Additional tests with co-transfected IDO1 shRNAs or integrin β4 signaling mutants showed a decrease in IDO1 expression and Kyn production as compared to control vector-transfected or WT β4 cells, respectively. Overall, we find that α6β4 signaling increased Trp metabolism through the Kyn pathway by transcriptionally upregulating IDO1 and increasing Kyn production in BT549 cells. These findings shed light on the role of integrin α6β4 in reprogramming Trp metabolism in TNBC and may help to explain why integrin α6β4 confers immunosuppression in TNBC.

Experimental Approach



Summary

- Whole genome bisulfite sequencing of BT549 EV and β4 cells shows that integrin α6β4 signaling increased gene body methylation and promoter demethylation of IDO1 gene versus EV cells.
- IFNG (IFNγ gene) strongly correlates with IDO1 gene expression.
- Gene set enrichment analysis shows that integrin α6β4 promotes an IFNγ response signature.
- Western blot and qPCR analysis shows increased IDO1 protein and gene expression in BT549 β4 cells, and further increased expression with IFNγ.
- Kynurenine production is greater in BT549 β4 cells than EV cells and is further increased with 24h IFNγ treatment.
- BT549 β4 cells with IDO1-specific shRNAs have decreased IDO1 expression and less kynurenine production with and without 24h IFNγ treatment as compared to PLKO.1 vector co-transfected cells.
- BT549 β4 plectin-binding and signaling domain mutant cells show modest alterations in IDO1 expression and kynurenine production compared to wild-type β4.

Background

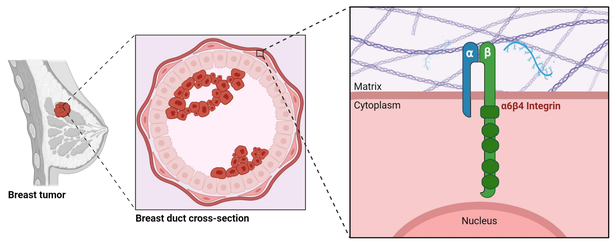


Figure 1: *Integrin α6β4 in TNBC.* Integrin α6β4 is a heterodimeric, transmembrane receptor responsible for nucleating cell-matrix adhesions. Integrin α6β4 is highly expressed in TNBC and basal-like breast cancer.

- The integrin α6β4 contributes to tumor survival, proliferation, angiogenesis, and metastasis in TNBC.
- The kynurenine (Kyn) pathway produces immunosuppressive intermediates and leads to *de novo* NAD^+ synthesis.
- The enzymes in the Kyn pathway are over-expressed in TNBC and confers the aggressiveness of this cancer.
- The cytokine IFNγ is present in the tumor microenvironment and promotes transcription of Kyn pathway enzymes, including IDO1.
- Preliminary findings indicate that α6β4 is associated with increased tryptophan (Trp) metabolism by upregulating IDO1 expression in TNBC.
- The role of integrin α6β4 in Trp metabolic reprogramming has yet to be investigated in TNBC.

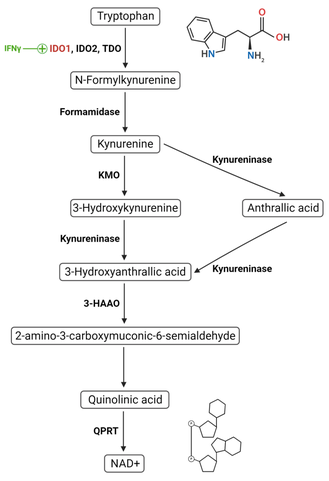


Figure 2: *Kynurenine Pathway.* The enzyme IDO1 degrades tryptophan to kynurenine and is upregulated by IFNγ cytokine signaling.

Hypothesis

Integrin α6β4 increases tryptophan metabolism through the kynurenine pathway by transcriptionally upregulating IDO1 expression in cooperation with IFNγ signaling.

Results

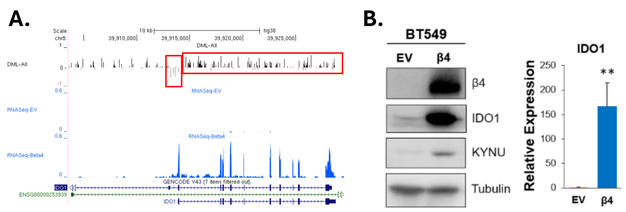


Figure 3: *Integrin α6β4 Stimulates IDO1 Expression in BT549 Cells.* **(A)** Whole genome bisulfite sequencing shows integrin β4 promotes gene body methylation of IDO1. **(B)** Results confirmed by western blot and qPCR.

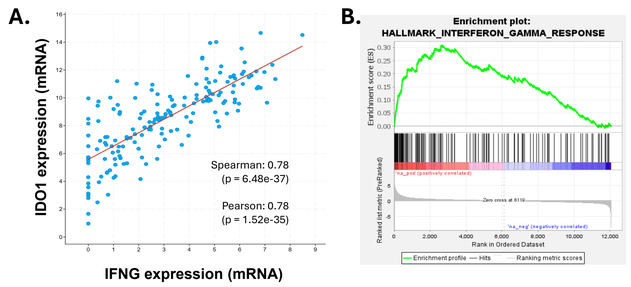


Figure 4: *IDO1 and Integrin α6β4 Associate with IFNγ and an IFNγ Response.* **(A)** TCGA PanCancer Atlas basal breast invasive carcinoma dataset reveals a strong correlation between IDO1 and IFNG expression. **(B)** GSEA of RNA sequence data shows that integrin α6β4 promotes IFNγ response signature.

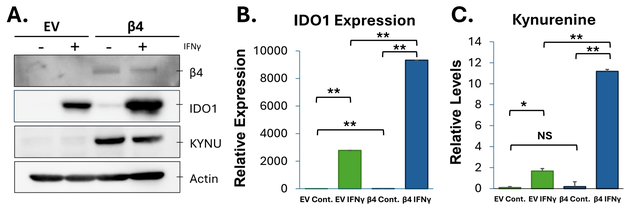


Figure 5: *IFNγ Preferentially Stimulates IDO1 Expression and Kynurenine Production Downstream of Integrin α6β4.* BT549 EV and β4 cells were treated with IFNγ for 24 hrs and then assessed for IDO1 by western blot **(A)** or qPCR **(B)** and kynurenine production **(C)**. * p< 0.0005, ** p<0.00001.

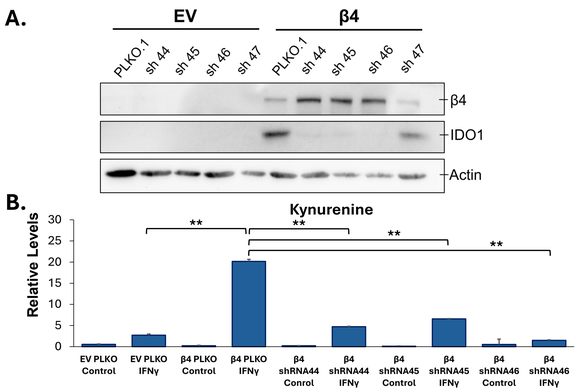


Figure 6: *IDO1 Suppression by shRNA Blocks Kynurenine Production.* IDO1 expression **(A)** and kynurenine production **(B)** is hindered in β4 cells with and without IFNγ stimulation with IDO1 suppression by shRNA. ** p<0.000001.

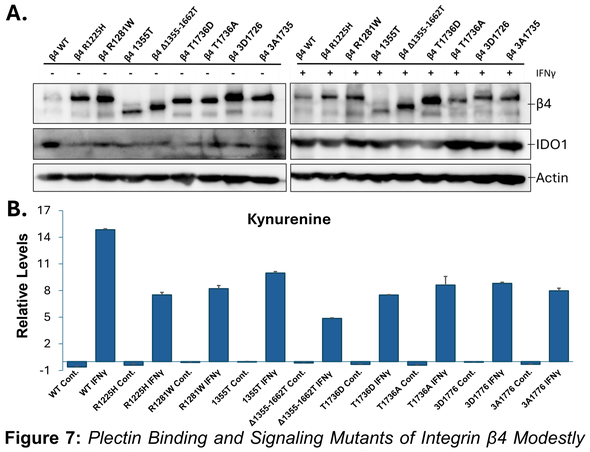


Figure 7: *Plectin Binding and Signaling Mutants of Integrin β4 Modestly Impact IDO1 Expression and Kynurenine Production.* Integrin β4 and IDO1 protein expression **(A)** and kynurenine **(B)** in BT549 cells expressing indicated mutants of integrin β4.

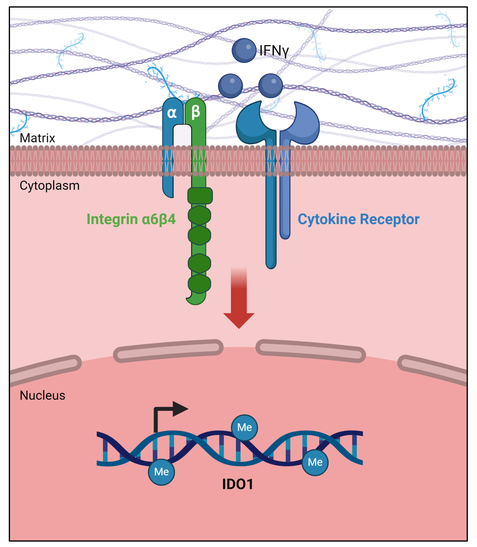


Figure 8: *Integrin α6β4 Primes IDO1 Gene for Increased IFNγ Response.* Integrin α6β4 methylates IDO1 gene body and demethylates promoter region, therefore promoting IFNγ-mediated IDO1 expression in TNBC.

Conclusions

In TNBC cells, integrin α6β4 primes the IDO1 gene by methylating the gene body and demethylating the promoter, which enhances the response signature of IFNγ to stimulate IDO1 transcription. Furthermore, the production of kynurenine is increased in BT549 β4 cells treated with IFNγ. Hence, integrin α6β4 plays a role in transcriptionally regulating the IDO1 gene to promote tryptophan metabolism through the kynurenine pathway.

Limitations and Future Directions

The project was limited by the 10-week duration of the research program, shipping delays in receiving reagents and use of one cell line to conduct our studies.

Future directions for this project are to test these conditions in other TNBC cell lines and to trace ^{13}C-Tryptophan through the metabolome using Stable Isotope Resolved Metabolomics (SIRM) to identify new routes of metabolic reprogramming in TNBC mediated by integrin α6β4.

References

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