Suppression of Triple-negative Breast Cancer Tryptophan Catabolism Requires Dual inhibition of TDO2/IDO1, but TDO2 Uniquely Affects Invasive Capacity

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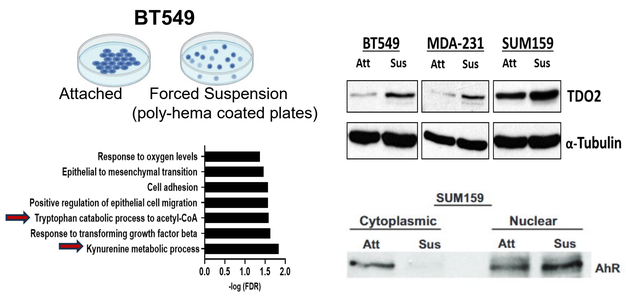
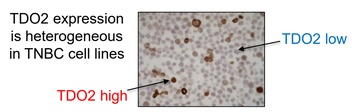
Background

- Triple-negative breast cancer (TNBC) has a rapid rate of metastatic recurrence (within 2-5 years post diagnosis) compared to other types of breast cancer. TNBC cell lines survive anchorage-independent conditions better than ER+ BC lines.
- We published that the enzyme Tryptophan 2,3-Dioxygenase (TDO2) increases in anchorage-independent TNBC cells (PMID: 26363006 and below). TDO2 catabolizes tryptophan (TRP) to kynurenine (KYN), which suppresses the immune system by expanding T regulatory cells and decreasing CD8+ T cell viability and function (PMID: 30143553).
- *TDO2* and a program of genes encoding immune-suppressive proteins are directly targeted by the microRNA-200 family (PMID: 30213797).
- AhR is located in the cytoplasm of attached SUM159PT, but is nuclear in SUM159PT in forced suspension culture (PMID: 26363006 and below))



Hypothesis

TRP catabolism affects TNBC invasive capacity in both an autocrine and paracrine manner and this pathway can be effectively targeted with a dual TDO/IDO inhibitor.



Results

IDO1 can Compensate for TDO2, but a Dual TDO/IDO Inhibitor Reduces Production of KYN and Downstream TRP Catabolites

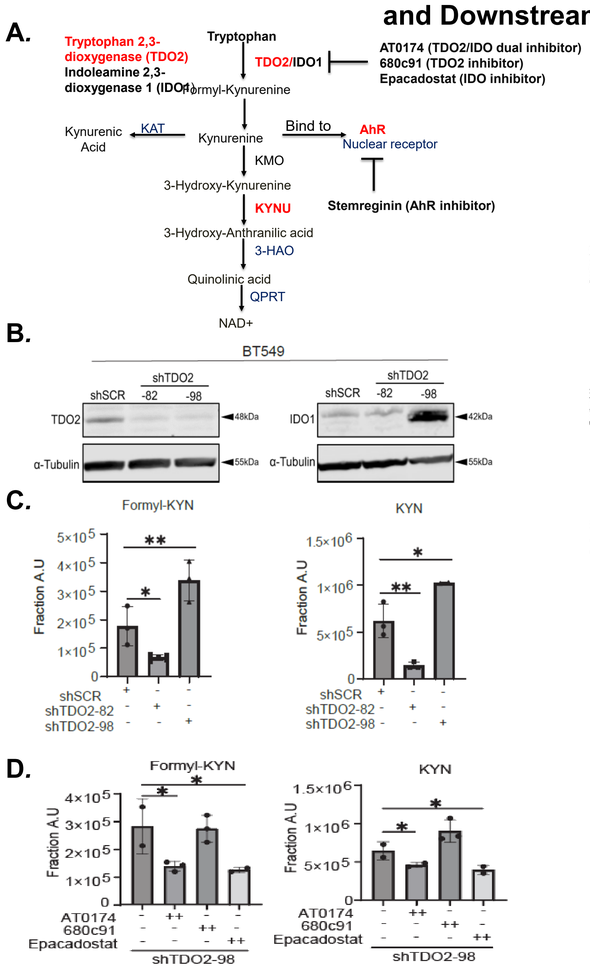


Figure 1. Knockdown of TDO2 increases IDO1. **A**. Tryptophan catabolism pathway **B**. Immunoblot of TDO2 and IDO1 expression **C.D.** The catabolite level fraction A.U (Abundance) of Formyl-Kynurenine (Formyl-KYN), and Kynurenine (KYN) as measured by mass spectrometry in condition media from BT549 with shTDO2 shSCR control or BT549 shTDO2-98 treated with 10μM AT0174, 680c91 or Epacadostat. Mean± SD with One-way ANOVA analysis *: p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

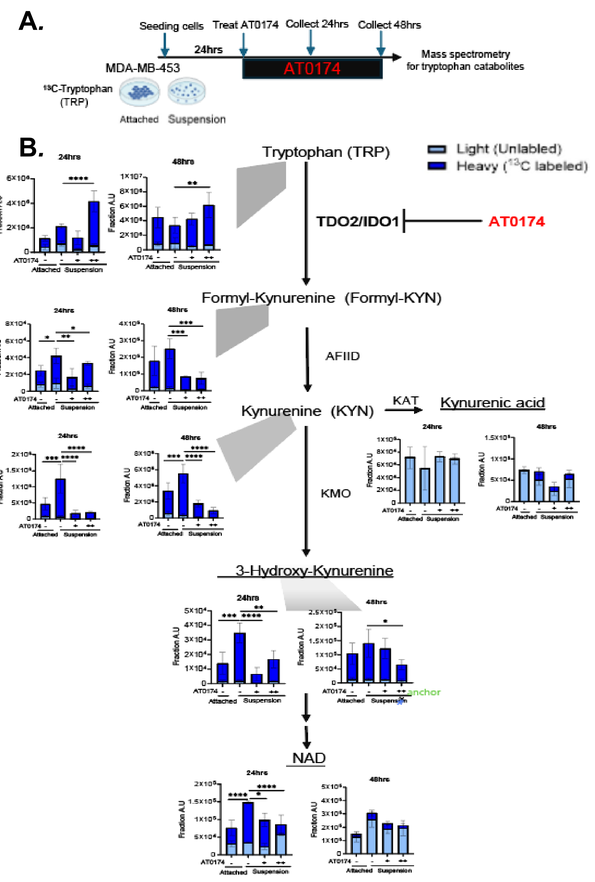


Figure 2. Tryptophan tracing with or without the dual TDO/IDO. A. Cells in attached versus forced suspension culture were incubated in tryptophan depleted media with ^{13}C labeled tryptophan (TRP) for 24 hours. Suspended cells were treated with vehicle (DMSO) or 1μM ("+") or 10μM AT0174 ("++") for 24 or 48 hours then harvested, lysed and analyzed by mass spectroscopy**. B**. Intracellular **heavy labeled** and **light (unlabeled)** TRP catabolites were measured and shown as Abundance Units (A.U). Biological triplicates were conducted for each group. Data displayed as mean ± SD with 2-way ANOVA analysis with p values *p<0.05, **p<0.01, **p<0.001, ****p<0.0001.

Results

Inhibition of TDO2/IDO1 or AhR reduces TNBC invasion and an epithelial to mesenchymal transition signature

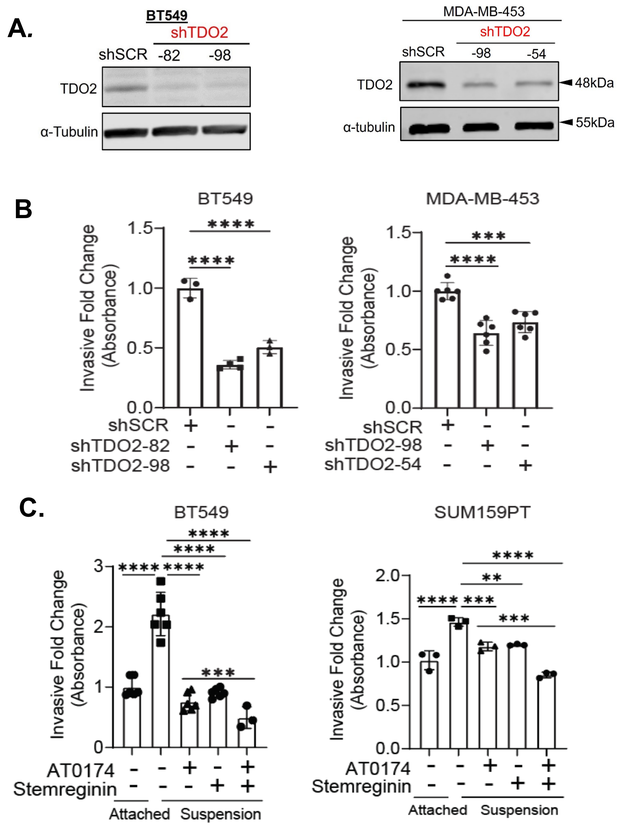


Figure 3. Knockdown or pharmacologic inhibition of TDO2 or AhR reduces TNBC invasion. **A.** BT549 and MDA-MB-45 with knockdown of *TDO2* (shTDO2) **B.** Invasion BT549 and MDA-MB-453 with scramble control (SCR) or TDO2 knockdown (shTDO2) was measured by transwell invasion through Cultrex for 24 hrs. **C.** BT549 or SUM159PT in attached or suspension condition with 10μM of TDO2/IDOi (AT0174) or AhRi (Stemreginin) for 48 hrs prior to measuring invasion for 24 hrs then stained with crystal violet, lysed and absorbance measured. Mean± SD with t-test or One-way ANOVA analysis *: p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

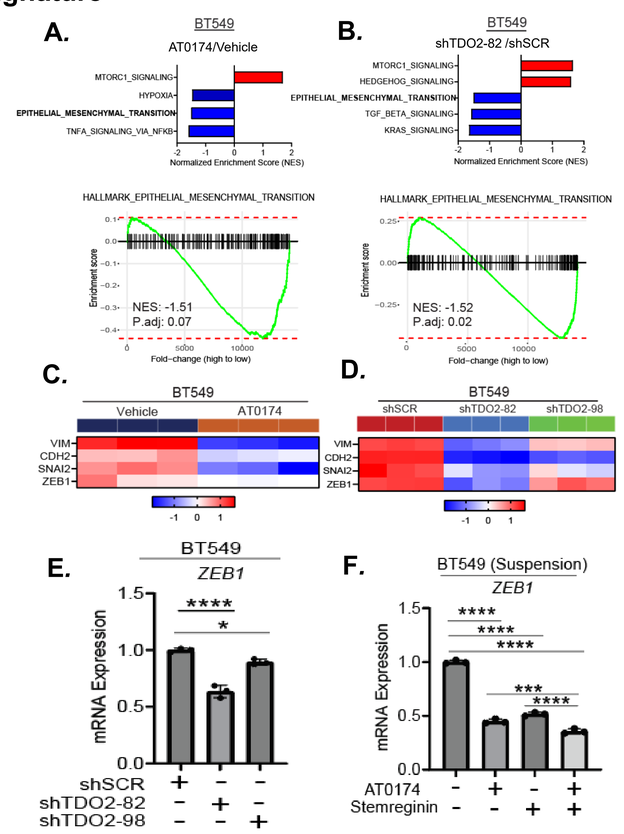
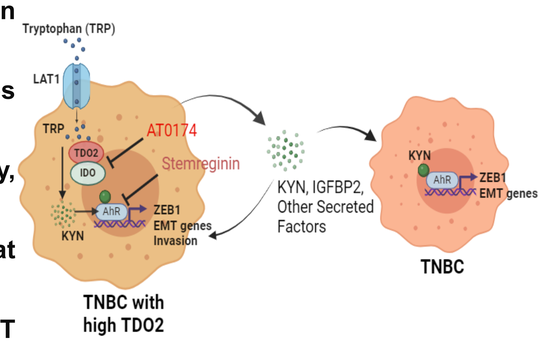


Figure 4. An epithelial to mesenchymal transition signature is reduced by Inhibition of TDO2 or AhR A, B. Gene sets enrichment analysis (GSEA) was used to analyze RNA-seq data from BT549 treated with AT0174 or vehicle for 48 hours and BT549 with *TDO2* knockdown (shTDO2) or control (shSCR) in biological triplicate. Pathways (p.adj <0.05) with negative and positive normalized enrichment scores (NES). **C, D.** RNA-seq for *VIM (Vimentin), CDH2 (N-Cadherin), SNAI2 (Slug)* and *ZEB1*. **E.** BT549 with shTDO2 and **F.** BT549 in suspension with vehicle, 10μM AT0174 or 10μM Stemreginin (AhRi) analyzed by one-way ANOVA *: p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions

- IDO1 can compensate to maintain TRP catabolism when TDO1 alone is targeted.
- However, the dual TDO2/ID01 inhibitor AT0174 decreases TRP catabolism.
- The TDO2/IDO1 dual inhibitor, or an inhibitor of AhR activity, reduces TNBC invasive capacity.
- IDO1 compensation does not affect invasion, indicating that TDO2 uniquely mediates invasion.
- Inhibition of TDO2 or AhR reduces invasion and an EMT gene signature.



Future directions

- Test TDO2/IDO1 inhibitor AT0174 in vivo in syngeneic mouse models (underway).
- Examine relative contribution of inhibition of TRP catabolism on tumor cells versus the immune microenvironment (TIME).
- Identify other secreted factors (in addition to KYN) made by anchorage-independent TNBC cells with high TDO2 activity that affect the TIME.

Acknowledgments and Funding

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