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 catalyzes tryptophan (TRP) to kynurenic (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). TDO2 catabolizes tryptophan (TRP) to quinolinic acid (QPA) or 3-hydroxy-kynurenine (3-HK) with nuclear AhR transcription factor (AhR) (PMID: 29786964, 29610610).
• Stemreginin (AhR inhibitor) suppresses TRP catabolism in forced TNBC cells (PMID: 30213797).

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

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

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 or shSCR control or BT549 shTDO2-98 treated with 10μM AT0174, 680c91 or Epacadostat. Means SD with One-way ANOVA analysis *: p<0.05, **p<0.01, ***p<0.001.

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 1C 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.

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-45 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/IDO (AT0174) or AhR (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 set 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 positive and negative 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 (AhR) 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/IDO1 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.

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