Targeting Interleukin-27 Receptor α in Murine HGSC Cells
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Abstract
In this study, we utilized a novel explainable artificial intelligence (XAI) approach to identify Interleukin-27 Receptor α (IL-27Rα) as a potential therapeutic target for high grade serous ovarian cancer (HGSC) patients based on the Cancer Genome Atlas (TCGA) dataset. High expression of IL-27Rα was found to be significantly correlated with poorer survival in HGSC patients. To investigate its therapeutic potential, we silenced IL-27Rα in murine HGSC cells using siRNA and assessed its impact on tumor proliferation, migration, invasion, and angiogenesis. Our results demonstrated that IL-27Rα siRNA effectively reduced its expression, as confirmed by Western Blot analysis, and significantly attenuated proliferation by clonogenic assay, migration by wound healing assay, and invasion assay, and angiogenesis by tube formation. These findings support the potential of targeting IL-27Rα as a promising strategy for improving outcomes in HGSC.

Introduction
HGSC has the highest mortality rate among gynecological cancers with 152,000 deaths out of 300,000 confirmed cases each year. The large mortality of HGSC can be attributed to the lack of symptoms until it has progressed to the later stages. Peritoneal spread is the leading cause of death from gynecologic cancers and is associated with abdominal obstruction, intestine, blood vessel obstruction. HGSC first metastasizes to the pelvic organs and blood (Stage 2), then the abdomen (Stage 3), and then beyond (Stage 4).

Artificial Intelligence (AI) is a set of algorithms that are capable of learning without human input and can be used to make new predictions through machine learning. AI was used to determine relationships between HGSC patients and the tumor microenvironment by using the TCGA database to identify potential therapeutic targets. An Explainable Artificial Intelligence (XAI) model was generated to interpret the results. According to the XAI model, IL-27Rα is a potential therapeutic target in HGSC patients because the high expression is associated with a lower 5-year survival rate.

Interleukin-27 Receptor (IL-27R) is a heterodimeric cytokine receptor composed of Interleukin-27 Receptor α (IL-27Rα) and glycoprotein 130. IL-27 is shown to display pro-tumour activity. It induces the expression of immune regulatory molecules such as IDO and PD-1, which leads to immune suppression through decreased T cell response and function, and eventually to tumor cell survival and proliferation. Silencing IL-27Rα may be a possible approach to improve survival rates among HGSC patients.

Preliminary Data

Figure 1. Shapley values graph explaining gene expression in HGSC patients using the TCGA database.

Hypothesis
Silencing IL-27Rα expression leads to anti-tumor activity in murine HGSC cells.

Objectives
• To determine the effect of silencing IL-27Rα in murine HGSC cells
• To examine the effect of silencing IL-27Rα on tumor migration, invasion, and colony formation in murine HGSC cells and angiogenesis in endothelial cells

Methods
TCGA Analysis: TCGA Database was used to generate a Kaplan-Meier survival curve using the database of HGSC patients (GEPIA).

Cell Lines: Murine HGSC cell lines ID8 and BR-Luc were cultured in Dulbecco modified Eagle High Glucose Medium and supplemented with 10% FBS. ID8 was additionally supplemented with 1% penicillin/streptomycin, 0.05% Insulin ST, while BR-Luc with 0.1% gentamicin. Cells were incubated at 37°C at 5% CO2. Human endothelial cell line R2F2 were cultured in MEM medium with 10% FBS, sodium pyruvate, nonessential amino acids, minimal essential medium vitamins, and glutamine.

Transfection: The IL-27Rα siRNA was synthesized by Sigma-Aldrich. Cells were transfected with IL-27Rα siRNA or non-silencing control siRNA. All siRNA transfections were carried out with HiPerfect (Qiagen) according to the manufacturer’s recommended protocol.

Western Blot Analysis: Cells were collected and lysed in RIPA buffer with protease and phosphatase inhibitors. Protein was quantified using BCA analysis and samples were run on 4-15% polyacrylamide SDS gel (Bio-Rad). Protein was transferred to polyvinyl difluoride membrane (Millipore) and blocked for 1h in 6% milk. Primary antibodies rat IL-27Rα (R&D Systems), mouse β-actin (Cell Signaling Technology), and rabbit p-SRC (Invitrogen) were prepared in 5% milk. Membranes were incubated overnight at 4°C with respective antibody. Secondary antibodies anti-rat IgG HRP (ThermoFisher), anti-mouse IgG HRP (Cell Signaling Technology), and anti-rabbit IgG HRP (Cell Signaling Technology), were prepared in 5% milk and membranes were incubated for 1h at 20°C. Bound antibodies were visualized using chemiluminescent and colorimetric detection.

Clonogenic Assay: Cells were plated onto 6-well plates with 2000 cells per well and then transfected with control siRNA or IL-27Rα siRNA after 24hr. Plates were incubated for 5-6 days. After incubation, the colonies were washed with PBS, stained with crystal violet, washed until excess crystal violet was removed, and quantified using ImageJ.

Wound Healing Assay: ID8 and BR-Luc were plated onto 6-well plates for 24 h before transfection with IL-27Rα siRNA or control siRNA. After transfection, cells were incubated until they reached 100% confluence. Each well was vertically scratched using a 230 pipet tip, and cellular debris was removed by washing with PBS. Images were captured at 0, 6, 12, 24, and 48 hours depending on cell line. Migration area was calculated by measuring the total distance that transfected cells migrated and is expressed as percentage migration.

Matrigel Invasion Assay: Transwells (Fisher) were coated with Matrigel (BD Biosciences) onto a 6-well plate. ID8 and BR-Luc cells transfected with control siRNA or IL-27Rα siRNA were suspended in serum-free medium and added into the upper Matrigel-coated transwells. Medium with 10% FBS was added to the lower wells. The plates were incubated for 48hr. The cells were then stained with the HEMA 3-stain system (Fisher) and were counted by using ImageJ software.

Tube Formation Assay: 10 µL of Matrigel (BD Biosciences) were added to each well of a 15-well angiogenesis plate and incubated for 1hr. RF-24 cells were seeded and transfected with either control or IL-27Rα siRNA for 50 µL of cell resuspension was added onto the Matrigel. The plates were incubated, and tube formation was observed at 16 hours.

Results

High IL-27Rα expression is correlated with lower survival rates of HGSC patients

Figure 2. (A) IL-27Rα is overexpressed in HGSC tissue compared to normal tissue. (B) High expression of IL-27Rα is associated with lower survival rate in HGSC patients.

Silencing IL-27Rα by siRNA leads to decreased expression of IL-27Rα in ID8 murine HGSC

Figure 3. (A) Silencing of IL-27Rα by Western Blot and (B) Densitometric analysis of IL-27Rα expression of Western Blot

Silencing IL-27Rα expression leads to decreased proliferation in murine HGSC

Figure 4. (A and B) Silencing of IL-27Rα in BR-Luc and ID8 clonogenic assays (C and D) and quantification of proliferation

Silencing IL-27Rα expression leads to decreased migration in murine HGSC

Conclusion
We have shown that IL-27Rα is associated with poor survival in patients with HGSC. Silencing of IL-27Rα using siRNA in murine HGSC cells reduced colony formation, migration, invasion and tube formation. IL-27Rα is possible therapeutic target for HGSC patients.

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References
5) Data was generated by the TCGA Research Network at: https://www.cancer.gov/tcga.