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TITLE: Assessing the Preclinical Efficacy of Combined Ferroptotic Agent and Secretory TRAIL-Armed NK Cells for Pseudomyxoma Peritonei

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CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center, West Hollywood, CA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Pseudomyxoma peritonei (PMP) is a rare cancer with an estimated incidence of 1–2 out of a million. It usually starts in the appendix. Sometimes it starts in another part of the bowel, the bladder, or the ovaries, but this is rare. Clinically, PMP usually presents with a variety of unspecific signs and symptoms, including abdominal pain and distention, ascites, or even bowel obstruction. Over the past two decades, oncologists have introduced extensive cytoreductive surgery (CRS) followed by hyperthermic intraperitoneal chemotherapy (HIPEC) as a novel treatment. Although CRS with HIPEC has become the treatment of choice for resectable PMP and has improved the survival of these patients, complete responses have been rare, and recurrences are common due to remaining residual microscopic disease. Thus, we need to develop a novel second-line therapy to improve the efficacy of current PMP therapy.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

In this grant application, we propose developing a second-line therapy to treat rare cancer pseudomyxoma peritonei (PMP). A combinatorial treatment comprised of secretory TRAIL-armed NK cells and the ferroptotic agent artesunate (ART) can be used as a second-line therapy to control residual microscopic disease of PMP after a first-line therapy (cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC).

2. KEYWORDS:

Pseudomyxoma peritonei Ferroptosis Apoptosis Synergistic interaction integration of death signals humanized patient-derived xenograft (PDX) mouse model tumoroids artesuante NK cells

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The specific aims of this project are to: (1) Assess the genetic signatures of tumor tissues from PMP patients, establish three-dimensional *ex vivo* tumoroid cultures of PMP, and examine the crosstalk between the ART-associated endoplasmic reticulum (ER) stress response pathway and the TRAIL-associated mitochondrial pathway in the synergistic induction of apoptosis during TA treatment (secretory TRAIL-armed NK cells + ART) in tumoroid cultures, and (2) Establish a humanized patient-derived xenograft (PDX) mouse model and assess the preclinical efficacy of the TA treatment in PDX tumors.

Major Task 1: Investigate the mechanism of the synergistic induction of apoptosis caused by the integration of signal transduction pathways in tumoroids of pseudomyxoma peritonei (PMP) (1-4 month)

Subtask 1: Determine the status of TRAIL receptors in PMP

Major Task 2: Generation of secretory TRAIL-armed NK cells (1-4 month)

Subtask 1: Isolate NK cells from the blood
Subtask 2: Generate secretory TRAIL-armed NK cells
Subtask 3: Expand secretory TRAIL-armed NK cells

Major Task 3: Determination of synergistic interaction (1-9 month)—50% completion in December 2022.

Subtask 1 Establish PMP tumoroid cultures with TRAIL receptor-positive tumor tissues
Subtask 2: Assess the biological response to artesunate and secretory TRAIL-armed NK cells in PMP tumoroides.
Subtask 3: Investigate mechanism of synergistic interaction between artesunate and secretory TRAIL-armed NK cells in PMP tumoroides.

Major Task 4: Establish PDX tumors and determine the growth and regression of PDX tumors during multimodal treatment (2-12 month).

Subtask 1: Establish humanized PDX mouse model with TRAIL receptor-positive tumor tissues.
Subtask 2: Examine preclinical efficacy of combinatorial treatment (artesunate + secretory TRAIL-armed NK cells) on PDX tumors.
Subtask 3: Statistical analysis of data

Establishment of organoid: Tumor tissues from patients were used to establish 3 D culture. Tumor tissue fragments were washed and incubated in chelating solution supplemented with EDTA. After incubation, tissues were washed with basal culture medium and resuspended in Basement Membrane Matrix. The Basement Membrane Matrix was overlaid with Human Stem Cell medium.

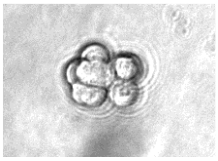

Figure 1. *Ex vivo* primary organoid cultures

Synergistic interaction between ferroptosis and apoptosis: We investigated the mechanism of crosstalk between ferroptosis and apoptosis. Data from our preliminary studies show that a treatment of PMP culture cells and established cancer cells combined with the ferroptotic agent ART and TRAIL markedly enhances TRAIL-induced cytotoxicity (**Fig. 2A**), in particular apoptosis (**Fig. 2B**). These synergistic effects were due to increased activation (cleavage) of caspase-3, which resulted in increased PARP cleavage, the hallmark feature of apoptosis (**Fig. 2B**). The combined treatment of TRAIL and ART did not enhance ferroptotic agent-induced lipid peroxidation, the hallmark feature of ferroptosis (**Fig. 2C**).

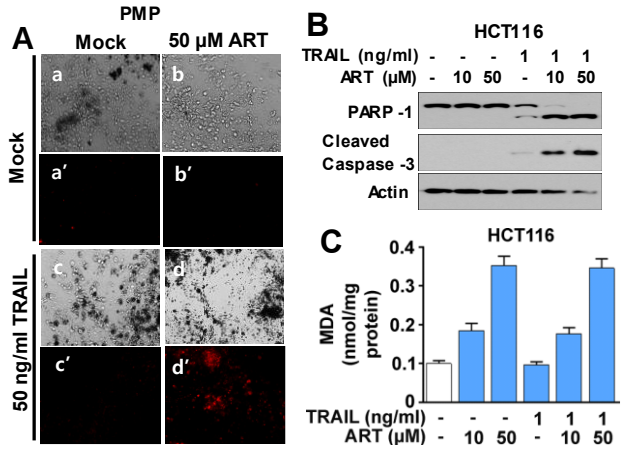

Figure 2. Effect of ART on TRAIL-induced apoptosis. Human PMP culture cells (**A**) and human colorectal carcinoma HCT116 cells (**B, C**) were pretreated with ART for 20 h and treated with TRAIL for 4 h in the presence of ART. (**A**) The cells were stained with propidium iodide. Phase-contrast images or fluorescent images were visualized under a light (upper panels) or fluorescence microscope (lower panels), respectively. (**B**) The cleavage of caspase-3 and PARP was detected using immunoblotting. Actin was used as a protein loading control in each lane. **(C)** Lipid peroxidation levels were analyzed using malondialdehyde (MDA) assay. MDA levels were determined and plotted. Error bars represent the mean ± SD from triplicate experiments. For statistical analysis, Student's t-test (two-sided, paired) was used. p-values: *, 0.05; **, 0.01.

Conclusion: Ferroptosis is considered a distinctive form of cell death compared to other types of death such as apoptosis. It is known to result from iron-dependent accumulation of lipid peroxides rather than caspase activation. However, we observed that ferroptosis interplays with apoptosis in PMP culture cells. In this grant period, we investigated a possible mechanism of this interplay between ferroptosis and apoptosis. Results from our studies reveal that combined treatment of the ferroptotic agent artesunate and the apoptotic agent TRAIL effectively enhanced TRAIL-induced apoptosis by increased caspase activation.

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

(1) We will obtain tumor tissues from PMP patients and identify transcriptional signatures of genes (TRAIL receptors). We will employ humanized triple transgenic NSG™-SGM3 mice for establishing patient-derived xenograft (PDX) mouse models with TRAIL receptor-positive tumor tissues. We will then assess the preclinical efficacy of multimodal treatment (Secretory TRAIL-armed NK cells + ferroptotic agent ART) on the growth and regression of PDX tumors.

(2) We will examine the mechanism of interaction between ferroptosis and apoptosis. We hypothesize that an increased caspase activation is due to disruption of mitochondrial membrane potential (ΔΨm). The alterations of mitochondrial membrane potential are probably due to an increase in oligomerization of BAX and its accumulation at the mitochondria during treatment with artesunate and TRAIL. This possibility will be investigated PMP cancer organoids.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Yong J. Lee, PI, translocated from the University of Pittsburgh to the Cedars-Sinai Medical Center. Due to movement, lab had been closed and performance has been slow down.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Yong J. Lee—No change
Name: Alexandra Gangi—No change
Name: Xuemo Fan—No change
Name: Stephen Shiao—No change
Name: Moura Tighiouart—No change
Snigdha Bhowmick---postdoc fellow is hired.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

A collaborator—Dr. Dong-Hyun Kim, an Associate Professor at the Northwestern University, joins as a collaborator.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: