Recombinant Thrombomodulin Has an Antitumor Effect and Enhances the Sensitivity of Gemcitabine Treatment of Pancreatic Cancer via G-protein Coupled Receptor 15

Kenel Furukawa1,2, Jinhua Ling1, Yichen Sun1, Yu Lu1, Jie Fu1, Nathan Nguyen1, Pranavi Garlapati1, Paul J Chiao1

Cellular and Molecular Oncology, The University of Texas MD Anderson Cancer Center2, Houston, TX

Department of Surgery – The Jikei University School of Medicine1, Tokyo, Japan

Background
- Pancreatic Ductal Adenocarcinoma (PDAC) causes 90% of pancreatic malignancies.
- Gemcitabine (GEM) is the primary cytotoxic chemotherapy treatment for PDAC. However, the apoptotic efficacy of GEM is reduced because GEM increases the phosphorylation of p65 and ERK.
- Thrombomodulin (TM) has anti-inflammatory and cytoprotective effects via G-protein coupled receptor 15 (GPR15).
- Recombinant Thrombomodulin (rTM), comprised of extracellular regions of TM, is approved to treat disseminated intravascular coagulation (DIC) in Japan.

Hypothesis
- rTM enhances the inhibition effect of GEM on cell proliferation.
- rTM inhibits the cell proliferation dependent on GPR15 expression.
- rTM inhibits cell proliferation by decreasing natural and GEM-induced p65 and ERK phosphorylation.

Methods
- We executed a variety of experiments with the following hypotheses:
  1. Step 1: Quantify the GPR15 Expression in Pancreatic Cancer Cell Lines
  2. Step 2: Measure the Efficacy of rTM and GEM Treatment based on GPR15 expression
  3. Step 3: Determine the Mechanism Through Which rTM Inhibits Cell Proliferation and Enhances Gemcitabine-induced apoptosis
- Western Blot Analysis
- MTT and Cell Proliferation Experiments

Results
- We assessed the effect of rTM treatment on cell proliferation by evaluating cell viability of HPNE and PDAC cell lines via MTT assay. The test illustrated that rTM significantly inhibited cells with high GPR15 expression: PATC66 and PATC153LM.
- We analyzed the effect of GPR15 on the rTM treatment of PDAC cell lines with GPR15 knockdown. We illustrated that rTM enhanced the proliferation inhibition effect of GEM.
- We evaluated the effect of rTM and GEM's combination treatment on pancreatic tumor effect and minimal side effects, achieving approval of rTM as a chemotherapy drug less difficult.

Discussion
- In the presence of GPR15, rTM decreased the activation of conventional and Gemcitabine-induced NF-κB and ERK phosphorylation.
- rTM's enhancement of GEM's cytotoxicity and anti-tumor effect was dependent on GPR15, suggesting that GPR15 is a cell surface receptor.
- rTM suppressed PDAC cell growth by inhibiting thrombin-induced PAR1 and NF-κB activation.
- Since rTM is widely used in patients with DIC-induced poor bodily function to minimal side effects, achieving approval of rTM as a chemotherapy drug less difficult.

Conclusion
- rTM had a significant anti-tumor effect and enhanced GEM's cytotoxicity of pancreatic cancer cells by inhibiting NF-κB and ERK activation via GPR15.

Acknowledgements: This presentation is supported by the National Cancer Institute through the U54 CA096297/CA096300: King Fou Memorial Project.