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

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Background

- Pancreatic Ductal Adenocarcinoma (PDAC) causes 90% of pancreatic malignancies, with a 92% mortality rate
- 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

We executed a variety of experiments with the following hypotheses:

- rTM enhances the inhibition effect of GEM on cell proliferation
- rTM hinderance of PDAC cell proliferation is dependant on GPR15
- rTM inhibits cell proliferation by decreasing natural and GEM-induced p65 and ERK phosphorylation

Methods

Step 1
- Quantify the GPR15 Expression in Pancreatic Cancer Cell Lines
  - Western Blot Analysis

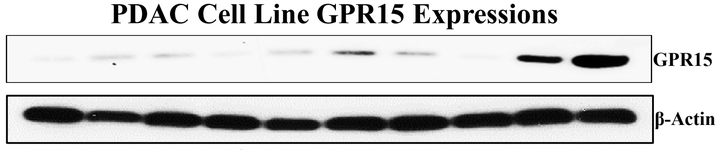
Step 2
- Measure the Efficacy of rTM and GEM Treatment based on GPR15 expression
  - MTT and Cell Proliferation Experiments

Step 3
- Determine the Mechanism Through Which rTM Inhibits Cell Proliferation and Enhances GEM-induced apoptosis
  - NF-KB and ERK Signal Pathway Analysis

Results

Step 1

PDAC Cell Line GPR15 Expressions

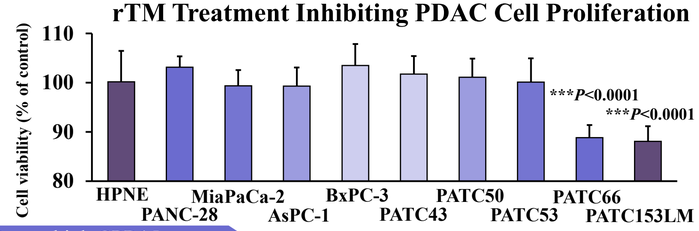


We performed a western blot analysis to determine the GPR15 expression in HPNE cells and 9 PDAC cell lines. PATC66 and PATC153LM cell lines yielded the highest GPR15 expression

Results

Step 2

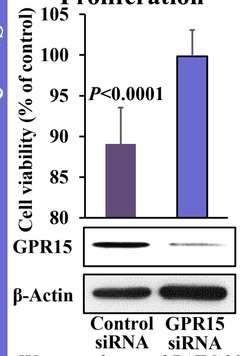
rTM Treatment Inhibiting PDAC Cell Proliferation



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

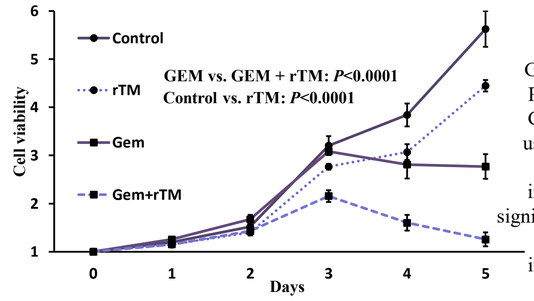
Due to high GPR15 expression, PATC66 was used for all following tests

GPR15 Mediation of rTM-Induced Inhibition of Cell Proliferation



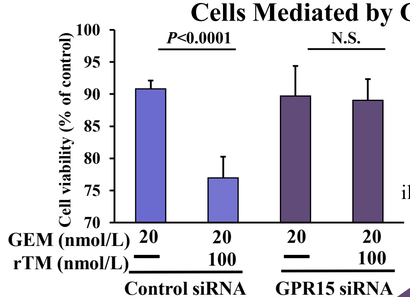
We treated control PATC66 cells and GPR15-knockdown PATC66 cells with rTM. In the GPR15 knockdown cells, rTM was unable to inhibit cell proliferation

rTM and GEM Treatment on Cell Proliferation



We evaluated the effect of rTM and GEM combined on PATC66 cells with GPR15 expression using a 5-day MTT assay. Results indicated that rTM significantly enhanced the proliferation inhibition effect of GEM

rTM and GEM Treatment on PDAC Cells Mediated by GPR15

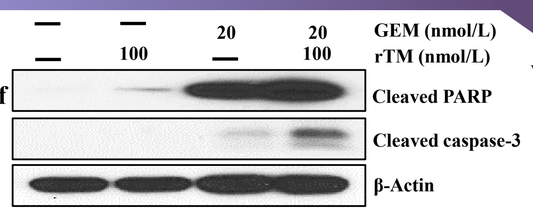


We analyzed the effect of GPR15 on rTM's enhancement of GEM with GPR15 knockdown PATC66 cells. The MTT assay illuminated that rTM enhanced GEM-induced cell inhibition only under GPR15 presence.

Statistical analysis consisted of non-paired t-tests, and ANOVAs

Step 3

rTM Enhancement of GEM-Induced Apoptosis

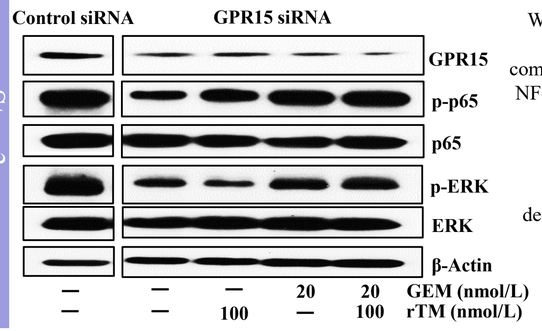


We used a western blot test to determine that rTM enhanced the apoptotic effect of GEM in PATC66 cells via PARP and caspase-9 cleavage

Results

Step 3

rTM Inhibition of GEM-induced NF-KB and ERK Phosphorylation via GPR15



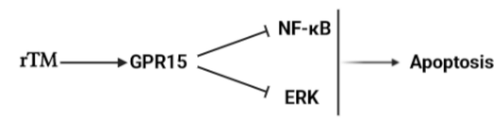
We evaluated the effect of the rTM and GEM combination treatment on NF-kB and ERK activity in PATC66 cells with GPR15 knockdown. Western blot analysis maintained that rTM decreased p65 and ERK phosphorylation only with GPR15 present

Discussion

- In the presence of GPR15, rTM decreased the activation of conventional and GEM-induced NF-KB and ERK phosphorylation.
- rTM's enhancement of GEM 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-KB 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 is less difficult

Conclusion

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



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