Abstract # 3522

Pre-treatment with FSH Enhances the Ability of a LHRH-Lytic Peptide Conjugate (EP-100) to Target and Destroy Human Pancreatic Cancer Cells

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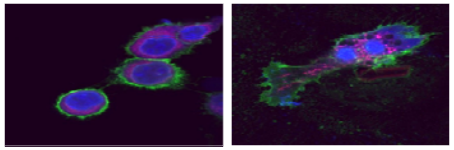
Background

Pancreatic Cancer

- One of the most lethal cancers with a 5-year survival rate less than 5%
- American Cancer Society estimated about 43140 new cases and 36800 deaths due to pancreatic cancer in 2010 [1]
- Gemcitabine extends the median survival time only by a few weeks [2] . There are no effective treatments
- A great need to develop effective therapies

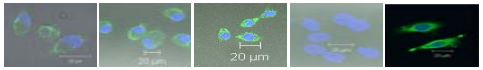
Lytic peptide conjugates with LHRH or βCG target and destroy breast, ovarian, prostate and testicular cancer cells [3,4,5,6]



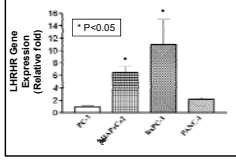
Untreated Cancer Cell | EP-100 Treated Cancer Cell @ 60 min

LHRHR is Expressed in Pancreatic Cancer Cells

Confocal Microscopy Using LHRHR Specific Antibody Demonstrates Presence of LHRH Receptor in Pancreatic Cancer Cells



MIAPaCa-2 | PANC-1 | BxPC-3 | Skov-3 | MDA-MB- 435S



Quantitative Real Time RT-PCR Demonstrates that Different Pancreatic Cancer Cells Express Different Numbers of LHRH Receptors

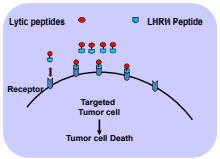
Hypothesis

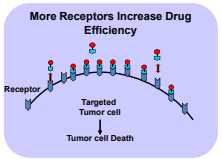
We hypothesize that pre-treatment with FSH will enhance the ability of a LHRH-lytic peptide conjugate (EP-100) to target and destroy pancreatic cancer cells by increasing LHRH receptor gene expression

Objectives

- To determine, in *in vitro* study whether pre-treatment with FSH increases the cytotoxicity of EP-100 in PANC-1 cells.
- To determine if pre-treatment of nude mice bearing PANC-1 cancer cell tumor xenografts with FSH enhances the ability of EP-100 to target and destroy these cells

Schematic Representation





Methods

***In vitro*:**

Quantitative Real Time RT-PCR: Total RNA was subjected to two-step SYBR Green real time RT-PCR using LHRH receptor and β2-m gene specific primers. Relative quantitation of LHRH receptor mRNA was performed by standard curve method. The graph represents relative fold expression of LHRH receptor gene in PANC-1 cells

Cell Viability Assay: Cell viability was determined using the MTT reagent method which is a colorimetric assay. The absorbance at 590 nm is directly proportional to the number of live cells. The cell viability is represented as % control.

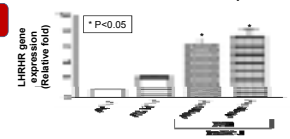
***In vivo*:**

PANC-1 tumor xenografts were generated in Athymic BALB/C female nude mice and were randomly distributed into the following groups (n=11): 1) Baseline controls, sacrificed at the beginning of the treatment (2) Vehicle treated controls (3) FSH treated (3 µg/day), s.c. for 3 days (4) EP-100 treated (0.02 mg/kg), i.v. (5) EP-100 treated (0.02 mg/kg), i.v.; pre-treated with FSH (3 µg/day), s.c. for 3 days prior to EP-100 (6) EP-100 treated (0.2 mg/kg), i.v. (7) EP-100 treated (0.2 mg/kg); pre-treated with FSH (3 µg/day), s.c. for 3 days prior to EP-100. Treatments (except FSH) were administered by tail vein injections given once a week for three weeks. Mice were necropsied one week following the last injection. Body weights, tumor weights and volumes (length x width^2)/2 were recorded and photographs of all the tumors were taken. At necropsy tissues were removed, weighed and fixed for histopathological studies.

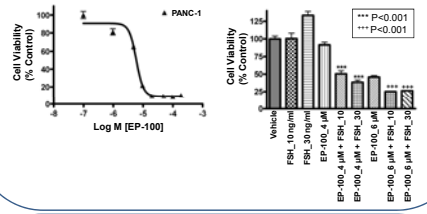
Results

FSH Increases LHRHR Gene Expression

In Vitro

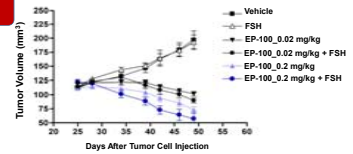


Pre-Treatment with FSH Decreases PANC-1 Cell Viability

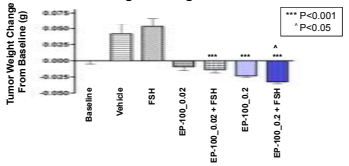


In Vivo

Tumor Xenograft Volume During Treatment

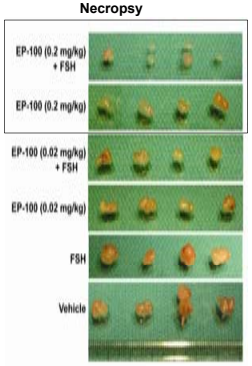


Tumor Weight Change From Baseline

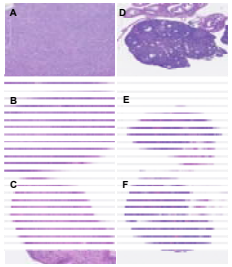


Results (continued)

Photographs of Tumors at Necropsy



Histopathology of Tumors and Ovaries



H & E stained tissue sections (5X magnification): (A-C) Tumor: A. Vehicle B. EP-100_0.2 mg/kg , C. EP-100_0.2 mg/kg + FSH; (D-F) Ovary: D. Vehicle E. EP-100_0.2 mg/kg and F. EP-100_0.2 mg/kg + FSH. Note the extensive cell necrosis in the tumors of the EP-100 and EP-100 + FSH treated mice and the hyper stimulation of follicle growth in the ovaries of the mice treated with EP-100 + FSH.

Conclusions

In Vitro

FSH increased the amount of LHRH receptor gene expression in PANC-1 cells by 3-fold and pre-treatment with FSH enhanced the cytotoxicity of EP-100

In Vivo

Pre-treatment with FSH significantly increased the efficacy of EP-100 *in vivo* at 0.02 mg/kg and 0.2 mg/kg compared to baseline

- FSH pre-treatment may be useful in treating pancreatic cancer with LHRH-lytic peptide conjugates

References

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