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14. ABSTRACT
Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with appallingly poor outcome. Recent years have witnessed the development of a number of combination therapies that have produced modest survival improvement but at the cost of increased adverse effects. We have recently demonstrated that pancreatic stellate cells (PSCs) secretes glutamine (Q) to promote the growth of pancreatic cancer cells (PCCs). I have also identified increased protein levels of pSTAT3 and survivin under conditions of Q-stimulation. Notably, Q-stimulated proliferation and pSTAT3 were attenuated by a novel compound called palmatine (PMT). Since both STAT3 and survivin's involved in therapeutic resistance to Gemcitabine (GEM) and Abraxane (Abr) in PDAC, we tested the hypothesis that Q-mediated signaling promotes PSC-PCC communication causes increased survival hallmarks of PDAC cells and that PMT can disrupt this communication to potentiate response to conventional therapeutics. Our results indicated that (i) PMT attenuated Q-mediated enhanced proliferation, clonogenicity and anchorage independent growth ability in multiple PDAC cell lines; (ii) PMT inhibits Q-mediated increased proliferation partially through STAT3; (iii) PMT reduces Q-induced increased Survivin's promoter activity through STAT3 and (iv) PMT works synergistically with GEM and Abr in different cell lines. Taken together, these data show potential clinical utility for the combination of PMT plus GEM in the treatment of pancreatic cancer.

15. SUBJECT TERMS
Pancreatic cancer; Therapeutic resistance; Palmatine; Glutamine; Pancreatic stellate cells; Gemcitabine; Abraxane

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TABLE OF CONTENTS

1. INTRODUCTION:

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with dismal prognosis. Due to occupational exposure, military personnel are at higher risk of developing and dying from PDAC. Gemcitabine (GEM) or GEM-based combination chemotherapy, mainly GEM plus Abraxane (Abr), are the standard-of-care first line treatment for PDAC, however, improvement on survival has been modest at the cost of increased adverse effects. Moreover, development of therapeutic resistance remains a major challenge limiting the effectiveness of treatment. It has been established that pancreatic stellate cells (PSCs) facilitate the growth of pancreatic cancer cells (PCCs) through their contribution in desmoplasia. Additionally, PSCs also stimulate cancer growth through various secretion factors. However, very few therapies target the interplay between PSCs and PCCs, which represents a novel therapeutic option. Efforts in Dr. Kumar's lab have identified that PSCs secrete glutamine (Q) that enhances proliferation of PCCs. I have also identified increased protein levels of pSTAT3 and survivin under conditions of Q-stimulation and that the observed effects were attenuated by a novel small molecule called palmatine (PMT). Since both STAT3 and survivin are associated with resistance to GEM and Abr, the **purpose** of this research is to decipher the roles of STAT3 and survivin in Q-mediated PSC-PCC communication, and to determine the effectiveness of PMT to inhibit this interplay and to potentiate response to standard of care. In this study, we are going to test the hypothesis that Q triggers PSC-PCC communication through STAT3/survivin upregulation to promote hallmarks of cancer and that PMT inhibits this process to improve response to conventional therapeutic agents GEM and Abr using cell culture and preclinical models.

2. KEYWORDS:

KEYWORDS: Pancreatic cancer; Therapeutic resistance; Palmatine; Glutamine; Pancreatic stellate cells; Gemcitabine; Abraxane

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

Major goals	**Completion**
1. Determine if PSC-secreted Q is responsible for the changes of cancer hallmarks in PCCs	20%
2. Determine the effects of PMT on Q release from PSCs and on cancer hallmarks in PCCs	50%
3. Establish the causal relationship between Q treatment, STAT3 and Survivin's promoter activity and verify the role of PMT in this process	80%
4. Determine the effectiveness of PMT to potentiate conventional therapy in PSCs and PCCs	90%

5. Determine the therapeutic activity of PMT with GEM plus Abr in preclinical model	5%

- **What was accomplished under these goals?**

Overall Accomplishments:

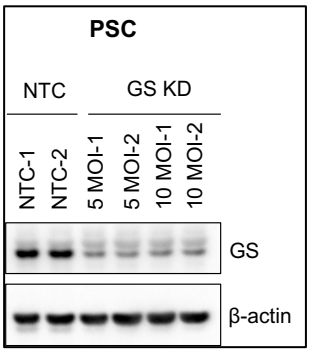

Figure 1. Validation of glutamine synthetase (GS) knockdown (KD) in pancreatic stellate cells (PSCs). PSCs were transducted with lentiviral particles carrying shRNA against non-targeted control (NTC) and Glutamine synthase (KD). Whole cell protein extracts prepared from NTC and KD cells were examined by Western Blot.

Overall I made significant progress in terms of (i) successfully completing some of the experiments related to both specific aims; (ii) mastering new techniques (CyTOF and metabolism experiments) and professional skills (writing and research presentations) (details discussed under *"What opportunities for training and professional development has the project provided?" and "PRODUCTS"* sections) (iii) help mentoring undergraduate/graduate rotation/medical students and (iv) learning to conduct experiments with rigor with consideration for inclusion of positive and negative controls and statistical analysis.

During Y1 funding, I have focused on (i) generating cell lines with stably STAT3 knockdown (KD) MIA PaCa-2 and PANC-1 cells and stable Glutamine Synthetase (GS) KD PSCs (Figures 1, 2 and discussed below); (ii) characterizing the STAT3 KD cell lines for their colony formation ability (Figure 3 and discussed below); (iii) optimization of conditions for studying glutamine (Q) effects on STAT3 signaling (Figures 4 and 5); (iv) examining the impact of PMT on STAT3 STAT3/survivin signaling using multiple cell lines (Figure 6); (v) functional significance of Q-induced changes (Figures 9-15). Additionally, I have tested and established that PMT works synergistically with GEM and Abr in different cell lines (Figures 17-19). Details discussed under each of the stated goals.

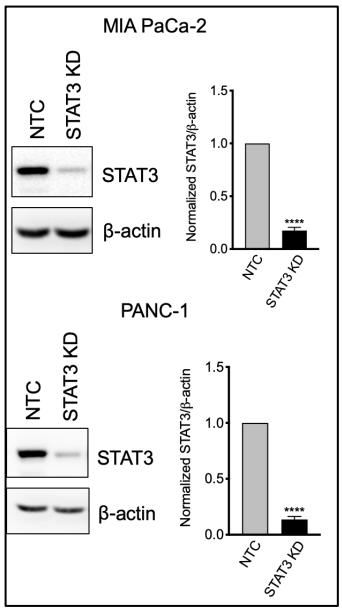

Figure 2. Validation of STAT3 knockdown (KD) in MIA PaCa-2 and PANC-1 cells. Stable STAT3 KD PCCs MIA PaCa-2 and PANC -1 were generated. PCCS were transducted with lentiviral particles carrying shRNA against non-targeted control (NTC) and STAT3 (KD). Whole cell protein extracts prepared from NTC and KD cells were examined by Western Blot.

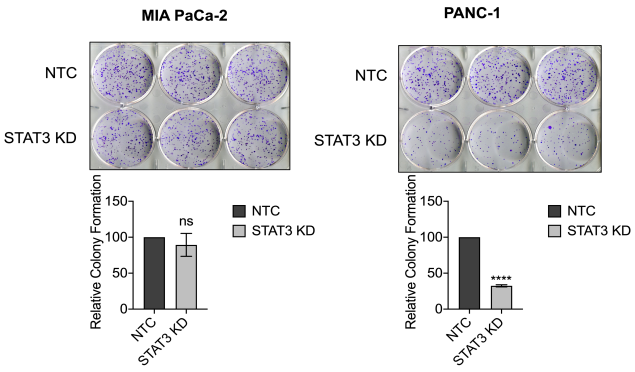

Figure 3. STAT3 KD significantly reduced colony formation ability in PANC-1 cells but not MIA PaCa-2 cells. 1000 (MIA PaCa-2) or 500 (PANC-1) NTC and STAT3 KD were seeded in each well of a 6-well plate in complete media. 8-14 days after seeding cells, colony formation was stained with crystal violet. Crystal violet staining was solubilized by 10% Acetic Acid and quantified by absorbance at 570nm.

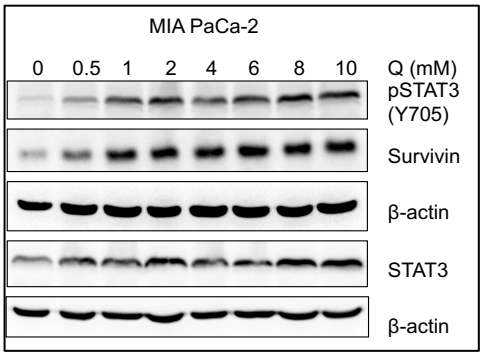

Figure 4. pSTAT3 levels increased with increasing concentrations of Q stimulation and saturated around 2 mM. Whole cell lysate from MIA PaCa-2 with 0-10 mM Q stimulation for 24h was prepared and used to analyze pSTAT3, STAT3 and survivin levels using western blotting.

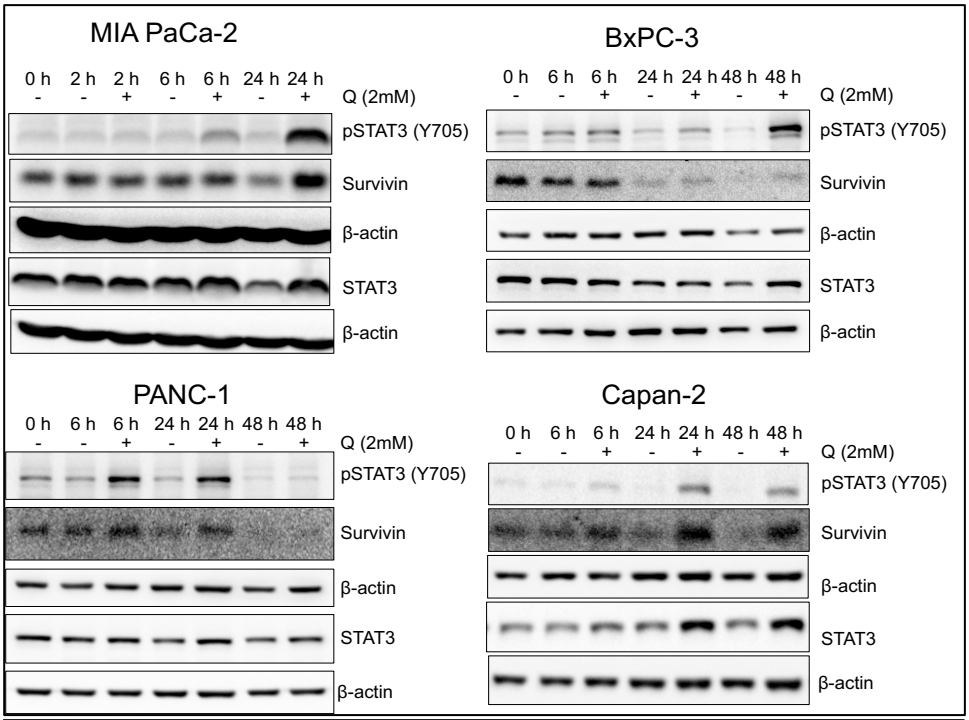

Figure 5. Q-stimulation induces pSTAT3 levels in various PCCs. Whole cell lysate from MIA PaCa-2, PANC-1, BxPC-3 and Capan-2 cells with or without 2mM Q stimulation was prepared and used to analyze pSTAT3, STAT3 and survivin levels using western blotting.

Additionally, I have standardized the conditions using CyTOF to evaluate alterations in markers associated with epithelial-to-mesenchymal transition (EMT) at single cell level and metabolic changes by measuring oxidative phosphorylation and mitochondrial respiration in response to glutamine in the presence and absence of PMT. These works are ongoing.

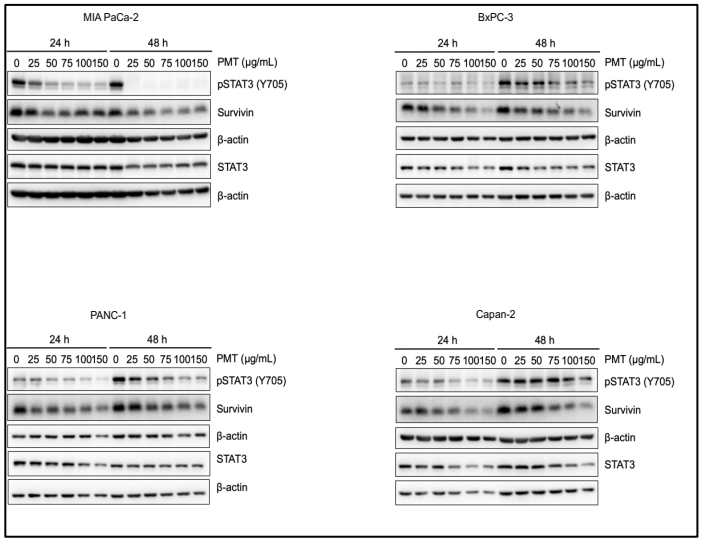

Figure 6. PMT reduces pSTAT3 and Survivn levels in multiple PDAC cell lines. Whole cell lysates from MIA PaCa-2, PANC-1, BxPC-3 and Capan-2 cells treated with 1-150 μg/ml PMT for 24 and 48 h were used to analyze pSTAT3, STAT3 and survivin levels with western blotting.

Major goal 1: Determine if PSC-secreted Q is responsible for the changes of cancer hallmarks in PCCs

To acquire conditioned media (CM) from PSCs with inhibited Q-release, I generated knockdown (KD) PSCs with shRNA against glutamine synthetase (GS). PSC-GS KD stable cell lines have been generated using shRNA and validated with Western Blot (Figure 1). Experiments are ongoing to generate CM from these cells to examine the impact on cancer cell hallmarks.

Major goal 2: Determine the effects of PMT on Q release from PSCs and on cancer hallmarks in PCCs

In PSC: to test the effect of PMT on Q-release from PSCs, I treated PSCs with increasing doses of PMT followed by measurement of Q concentration in the conditioned media (CM) and cell lysate with glutamine/glutamate-Glo Assay Kit (Promega, Madison, WI). Our results reveal that PMT treatment reduces secreted levels of Q in the CM but not intracellular levels. (Figure 7).

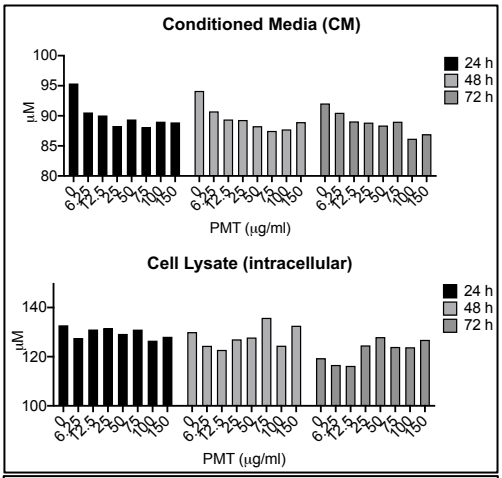

Figure 7. PMT reduces Q levels in PSC CM. PSCs were treated with increasing doses of PMT for 24h – 72h. CM and cell lysates were collected and measured following the manufacturer's instructions of Glutamine/glutamate-Glo Assay Kit (Promega, Madison, WI).

In PCCs:

To test the effect of PMT on cancer hallmarks of PCCs, treat PCCs with increasing concentrations of PMT followed by evaluation of cancer hallmarks. I have examined the impact of Q and PMT on cancer hallmarks including proliferation, colony formation ability, anchorage independent growth and EMT changes (through CyTOF).

a) Pancreatic cancer cells MIA PaCa-2, BxPC-3, PANC-1 and Capan-2 were treated with increasing concentrations of PMT for 48 h, and cell proliferation was determined by MTT assay. Data presented is an average + SD of 2 - 4 independent experiments conducted in triplicate. Statistical analysis was determined by one-way ANOVA. Results from MTT proliferation assay showed that PMT treatment significantly reduced proliferation in multiple PDAC cell lines (Figure 8).

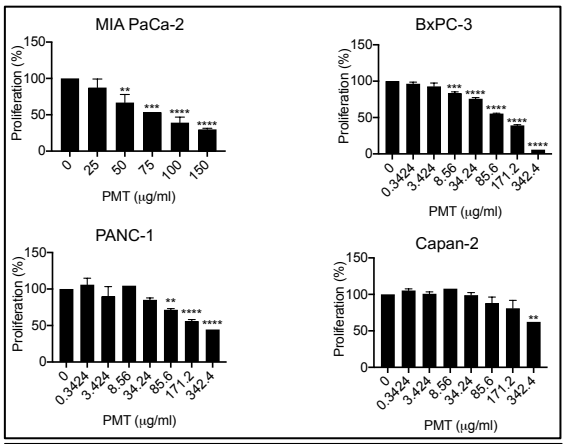

Figure 8. PMT inhibits proliferation of PCCs. Pancreatic cancer cells MIA PaCa-2, BxPC-3, PANC-1 and Capan-2 were treated with increasing concentrations of PMT for 48 h, and cell proliferation was determined by MTT assay.

b) The effect of PMT on Q-induced proliferation in PCCs: Pancreatic cancer cells MIA PaCa-2, BxPC-3, PANC-1 and Capan-2 were starved from Q and glucose overnight, then stimulated with 2 mM Q together with increasing concentrations of PMT for 48 h. Cell proliferation was measured by MTT assay. Data presented is an average + SD of 2 - 3 independent experiments conducted in triplicate. Statistical analysis was determined by one-way ANOVA. MTT proliferation assay in multiple PDAC cell lines showed that Q significantly induced proliferation in PDAC cell lines, which can be significantly suppressed by PMT treatment (Figure 9).

c) The effect of PMT on Q-induced anchorage independent growth: MIA PaCa-2 cells starved from Q and glucose overnight were stimulated with Q and treated with increasing concentrations of PMT for 24 h. Cells were then trypsinized and equal numbers of live cells from each treatment were seeded in soft agar. 7-10 days after seeding cells, colony formation was quantified following the manufacturer's instructions using CytoSelect 96-well Cell Transformation Assay (Cell Biolabs, San Diego, CA). Data presented is an average + SD of 3 independent experiments. Statistical analysis was

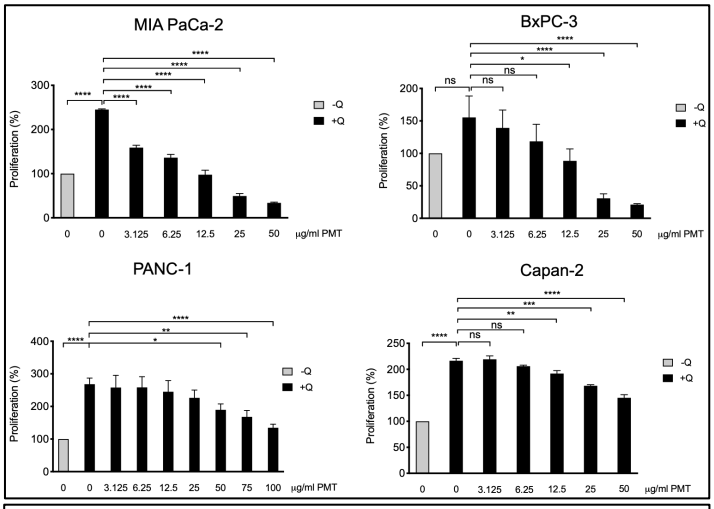

Figure 9. PMT suppresses Q-stimulation induced proliferation in PCCs. Pancreatic cancer cells MIA PaCa-2, BxPC-3, PANC-1 and Capan-2 were starved from Q and glucose overnight, and stimulated with 2 mM Q together with increasing concentrations of PMT for 48 h. Cell proliferation was measured by MTT assay.

determined by one-way ANOVA. soft agar assay in MIA PaCa-2 cells showed that Q stimulation significantly increased anchorage independent growth ability, which can be significantly reduced by PMT treatment (Figure 10).

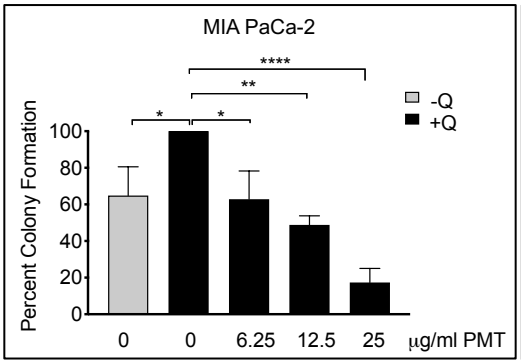

Figure 10. PMT inhibits Q-stimulation induced anchorage independent growth ability in MIA PaCa-2 cells. MIA PaCa-2 cells starved from Q and glucose overnight were treated with Q and increasing concentrations of PMT for 24 h. Cells were then trypsinized and equal numbers of live cells from each treatment were seeded in soft agar. 7-10 days after seeding cells, colony formation was quantified following the manufacturer's instructions using CytoSelect 96-well Cell Transformation Assay (Cell Biolabs, San Diego, CA).

d) The effect of PMT on Q-induced colony formation ability: PANC-1 cells starved from Q and glucose overnight were treated with Q and increasing concentrations of PMT for 48 h. Cells were then trypsinized and 500 live cells from each treatment were seeded in each well of a 6-well plate in complete media. Following 10 -14 days of incubation, colonies were stained with crystal violet. Stained cells were solubilized using 10% acetic acid and absorbance was measured at 570nm for quantification. Data presented is an average + SD of 3 independent experiments. Statistical analysis was determined by one-way ANOVA. Q stimulation significantly increased colony formation ability of PANC-1 cells. This increased clonogenic ability was not suppressed by PMT treatment (Figure 11).

<u>Major goal 3: Establish the causal relationship between Q treatment, STAT3 and Survivin's promoter activity and verify the role of PMT in this process</u>

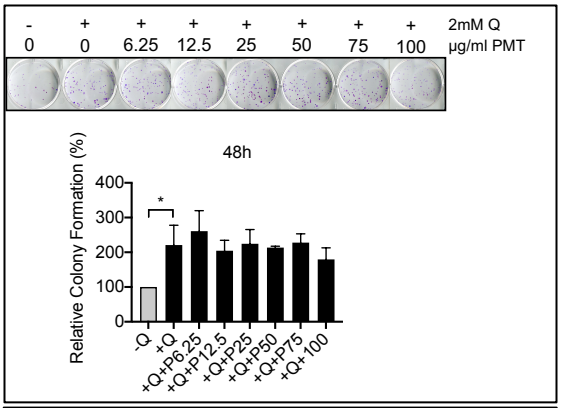

Figure 11. PMT does not inhibit Q-simulation induced proliferation in PANC-1 cells. PANC-1 cells starved from Q and glucose overnight were treated with Q and increasing concentrations of PMT for 48 h. Cells were then trypsinized and 500 live cells from each treatment were seeded in each well of a 6-well plate in complete media. Following 10 -14 days of incubation, colonies were stained with crystal violet. Stained cells were solubilized using 10% acetic acid and absorbance was measured at 570nm.

A. To study the role of STAT3 in Q-induced enhanced promoter activity of survivin. Generate STAT3 KD and NTC PCC cell lines with shRNA.

a. STAT3 KD stable cell lines MIA PaCa-2 and PANC-1 have been generated using lentiviral particles carrying shRNA against non-targeted control (NTC) and STAT3 (KD). 2 clones of NTC and 4 clones of STAT3 KD were generated. I have characterized these cell lines for efficacy of STAT3 KD using Western Blot (Figure 2). Their clonogenic ability was characterized with colony formation assay (Figure 3): 1000 MIA PaCa-2 or

500 PANC-1 NTC and STAT3 KD were seeded in each well of a 6-well plate in complete media. 8-14 days after seeding cells, colonies were stained with crystal violet. Stained cells were solubilized using 10% acetic acid and absorbance was measured at 570nm. Data presented is an average + SD of 3 independent experiments. Statistical analysis was determined by Student's t test. Our data suggests that STAT3 KD significantly reduced colony formation ability in PANC-1 cells but not MIA PaCa-2 cells.

b. In order to determine the role of STAT3 on Q and PMT-mediated change in proliferation, I have tested the impact of PMT on Q-stimulated proliferation in these NTC and STAT3 KD cells (Figure 12), MIA PaCa-2 and PANC-1 NTC and STAT3 KD cells were starved from Q and glucose overnight, and stimulated with 2 mM Q together with increasing concentrations of PMT for 48 h. Cell proliferation was measured by MTT assay. Data presented is an average + SD of 3 - 4 independent experiments. Statistical analysis was determined by Student's t test or one-way ANOVA. Our results showed that Q-stimulation enhanced proliferation in both NTC and STAT3 KD MIA PaCa-2 cells and PANC-1 cells (Figure 12 A-D), and that STAT3 KD cells were less sensitive to PMT treatment to reduce Q-induced proliferation in both MIA PaCa-2 (Figure 12E) and PANC-1 (Figure 12F) cells, indicating that PMT inhibits Q-mediated increased proliferation partially through STAT3.

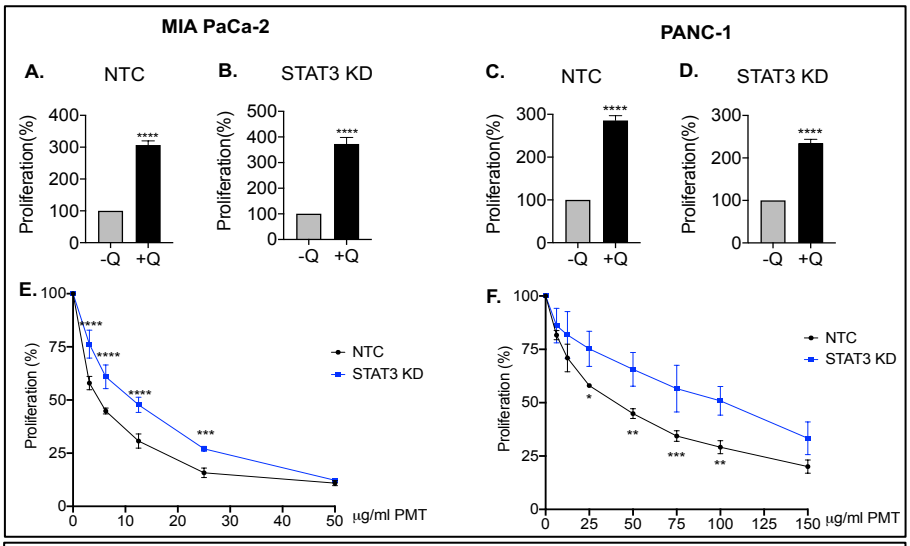

Figure 12. STAT3 KD PCCs (MIA PaCa-2 and PANC-1) are less sensitive to PMT treatment. MIA PaCa-2 and PANC-1 NTC and STAT3 KD cells were starved from Q and glucose overnight, and stimulated with 2 mM Q together with increasing concentrations of PMT for 48 h. Cell proliferation was measured by MTT assay. A – D represents response to Q stimulation in MIA PaCa-2 cells (A, B) and PANC-1 cells (C,D), E-F represents impact of PMT on Q-stimulation induced proliferation in MIA PaCa-2 cells (E) and PANC-1 cells (F).

B. To establish the causal relationship between Q and survivin activation

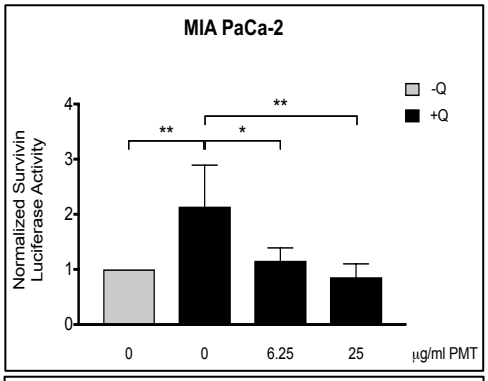

Figure 13. Q stimulation enhanced survivn's promoter activity, which can be inhibited by PMT. MIA PaCa-2 cells were transiently transfected with survivin-luciferase reporter and Renilla luciferase plasmids followed by treatment with and without Q and PMT for 6h. Survivin's reporter activity was measured with Dual Luciferase Reporter Assay system and Glomax Luminometer (Promega, Madison, WI).

I performed Luciferase Reporter Assay in PCCs MIA PaCa-2 and BxPC-3 to examine how Q and PMT affect Survivin's promoter activity. PCCs were transiently transfected with survivin-luciferase reporter and Renilla luciferase plasmids followed by treatments of Q with or without PMT for 6h. Survivin's reporter activity was measured with Dual Luciferase Reporter Assay system and Glomax Luminometer (Promega, Madison, WI). Data presented is an average + SD of 4 independent experiments. Statistical analysis was determined by one-way ANOVA. Our result in MIA PaCa-2 cells showed that Q stimulation significantly enhanced survivn's promoter activity, and PMT inhibited this process (Figure 13). I have experienced technical problems with transfection in BxPC-3 cells, but I will perform this experiment in other PDAC cell lines.

C. To test if Q-induced enhanced survivin promoter activity is caused by activation of STAT3.

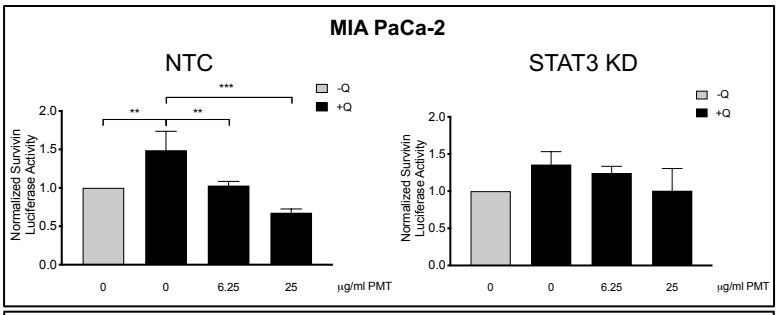

Figure 14. STAT3 KD MIA PaCa-2 cells are less sensitive to Q-stimulation and PMT-inhibition of survivin's promoter activity. MIA PaCa-2 NTC and STAT3 KD cells were transiently transfected with survivin-luciferase reporter and Renilla luciferase plasmids followed by treatment with and without Q and PMT for 6h. Survivin's reporter activity was measured with Dual Luciferase Reporter Assay system and Glomax Luminometer (Promega, Madison, WI).

I performed Luciferase Reporter Assay in STAT3-KD and NTC cells in PDAC cell lines MIA PaCa-2 and PANC-1. Cells were transiently transfected with survivin-luciferase reporter and renilla luciferase plasmids followed by treatments of Q with or without PMT. Survivin's reporter activity was measured with Dual Luciferase Reporter Assay system and Glomax Luminometer (Promega, Madison, WI). Data presented in MIA PaCa-2 cells is an average + SD of 3 independent experiments. Statistical analysis was determined by one-way ANOVA. Data presented in PANC-1 cells is a single experiment. Our data in MIA PaCa-2 cells demonstrated that Q stimulation

leads to significant induction of Survivin's promoter activity in NTC but not STAT3 KD cells and that 6.25 and 25 ug/ml PMT significantly reduced Q-induced Survivin's promoter luciferase activity in NTC but not STAT3 KD cells, indicating that PMT reduces Q-induced increased Survivin's promoter activity through STAT3 (Figure 14). I am currently repeating these experiments uisng PANC-1 cells. Interestingly, in PANC-1 cells both NTC and STAT3 KD cells showed increased levels of survivin's promoter luciferase activity with Q stimulation, however, survivin's promoter activity was not suppressed by PMT treatment (Figure 15). This is in part correlated to the observation that PANC-1 cells are less sensitive to PMT treatment to suppress Q-induced proliferation (Figure 9) and colony formation ability (Figure 11). This differential effect may be cell specific or related to genotypic differences.

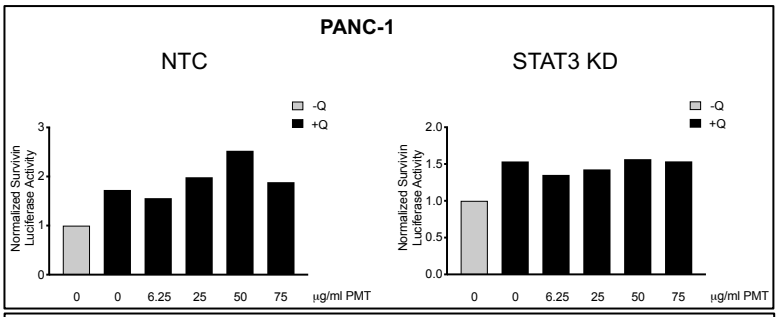

Figure 15. PMT does not inhibit Q induced increased survivin's promoter activity. PANC-1 NTC and STAT3 KD cells were transiently transfected with survivin-luciferase reporter and Renilla luciferase plasmids followed by treatment with and without Q and PMT for 6h. Survivin's reporter activity was measured with Dual Luciferase Reporter Assay system and Glomax Luminometer (Promega, Madison, WI).

D. Based on our exciting findings demonstrating the potential of PMT to inhibit Q-induced biological outcome and partial rescue with STAT3, in order to decipher the underlying mechanism (possibly in addition to STAT3), I performed RNA-seq analysis in Q-stimulated MIA cells in the presence and absence of increasing doses of PMT and identified differential expressed genes that are significantly associated with low survival in PDAC. These data are currently being analyzed.

Major goal 4: Determine the effectiveness of PMT to potentiate conventional therapy in PSCs and PCCs

A. To establish dose response of PSCs and PCCs to PMT, gemcitabine (GEM) and Abraxane (Abr), I tested the effect of these agents singly to determine IC50 values in various cell lines including PSCs and PCCs using MTT assay (Figure 16). PSCs and PCCs (MIA PaCa-2, BxPC-3, PANC-1 and Capan-2) were treated with increasing concentrations of PMT, GEM and Abr for 48 h, and cell proliferation was determined by MTT assay. Data presented is an average + SD of 2 - 4 independent experiments. Statistical analysis was determined by one-way ANOVA. The estimated IC50s of PMT are PSC: >100 µg/ml, MIA PaCa-2: 64.60 µg/ml, PANC-1: ~200 µg/ml, BxPC-3: ~90 µg/ml and Capan-2: >300 µg/ml. The estimated IC50s of GEM are PSC: 30.72 nM, MIA PaCa-2: >500 nM, PANC-1: >1 µM, BxPC-3:

32.69 nM and Capan-2: >1 μM. The estimated IC50s of Abr are PSC: 36.78 nM, MIA PaCa-2: ~25 nM, PANC-1: ~1 μM, BxPC-3: 33.53 nM and Capan-2: >1 μM.

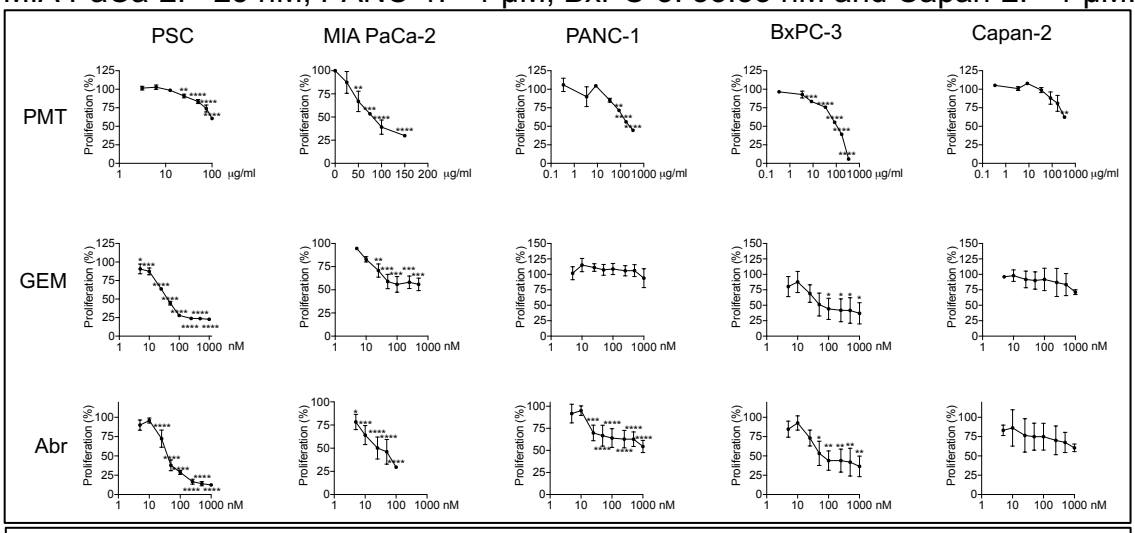

Figure 16. Dose response to PMT, GEM and Abr alone in PSCs and PCCs. PSCs and PCCs (MIA PaCa-2, BxPC-3, PANC-1 and Capan-2) were treated with increasing concentrations of PMT, GEM and Abr for 48 h, and cell proliferation was determined by MTT assay.

In order to determine the combinatorial benefits of PMT in addition to GEM and Abraxane *in vitro*, I tested the combination effect of these agents in various cell lines including PSCs and PCCs using MTT assay.

a) Logarithmically growing PSCs were treated with 100 μg/ml PMT, 25 nM GEM and 25 nM Abr alone, combination of GEM plus PMT, Abr plus PMT and combination of PMT, GEM and Abr for 48h. Cell proliferation was determined by MTT assay. Data presented is an average + SD of 3 independent experiments conducted in triplicate. Statistical analysis was determined by one-way ANOVA. In PSCs, PMT, GEM and Abr alone significantly reduced proliferation compared to control. Combining with PMT further significantly reduced proliferation compared to single treatment of GEM or Abr. Triple combination of PMT, GEM and Abr also significantly reduced proliferation from double combination GEM and Abr. Interestingly, PMT plus GEM showed no significant difference from triple combination of PMT, GEM and Abr,

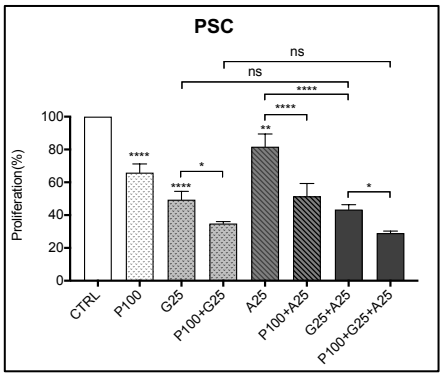

Figure 17. PMT potentiates growth inhibitory effect of conventional therapeutics in PSCs. PSCs were treated with 100 μg/ml of PMT, 25 nM GEM and 25nM Abr, combination of GEM plus PMT, Abr plus PMT and combination of PMT, GEM and Abr for 48h. Cell proliferation was determined by MTT assay.

suggesting that this double combination showed similar inhibitory effect as triple combination (Figure 17).

b) Logarithmically growing MIA PaCa-2 cells were treated with 50 µg/ml PMT, 50 nM GEM and 50 nM Abr alone, combination of GEM plus PMT, Abr plus PMT and combination of PMT, GEM and Abr for 48h. Cell proliferation was determined by MTT assay. Data presented is an average + SD of 3 independent experiments conducted in triplicate. Statistical analysis was determined by one-way ANOVA. In MIA PaCa-2 cells, PMT, GEM and Abr alone significantly reduced proliferation compared to control. PMT further significantly reduced proliferation when combining with GEM. Remarkably, double combination of PMT and Abr showed the best growth inhibitory effect. PMT and Abr combination also significantly reduced proliferation compared to the standard of care treatment GEM and Abr (Figure 18). We will be validating these observations in preclinical models as part of Major Goal 5.

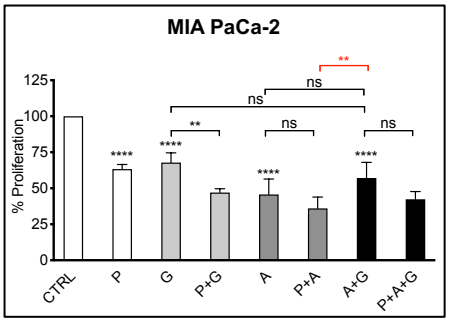

Figure 18. PMT potentiates growth inhibitory effect of conventional therapeutics in MIA PaCa-2 cells. MIA PaCa-2 cells were treated with 50 µg/ml of PMT, 50 nM GEM and 50 nM Abr, combination of GEM plus PMT, Abr plus PMT and combination of PMT, GEM and Abr for 48h. Cell proliferation was determined by MTT assay.

c) Intrigued by these combination effect in proliferation, I further explored the molecular mechanism of these combinations. I treated MIA PaCa-2 cells with 50 µg/ml PMT, 50 nM GEM and 50 nM Abr alone, combination of GEM plus PMT, Abr plus PMT and combination of PMT, GEM and Abr for 48h. Whole cell extracts were collected to performed Western Blot to investigate the role of pSTAT3 and survivin in these treatment conditions (Figure 19). Band intensity was quantified with Syngene Gene tools gel analysis software. Data shown is a representation of 3 independent experiments.

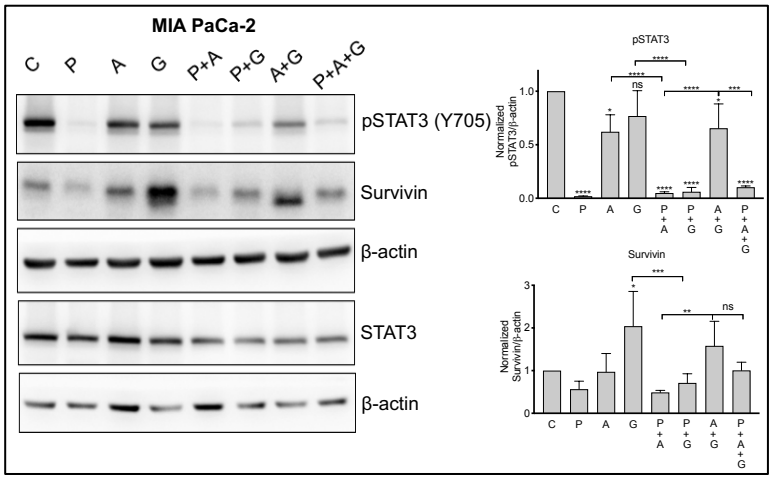

Figure 19. PMT reduces pSTAT3 and survivin levels from cells treated with GEM or Abr or GEM+Abr combination. MIA PaCa-2 cells were treated with 50 µg/ml of PMT, 50 nM GEM and 50 nM Abr, combinations of GEM plus PMT, Abr plus PMT and combination of PMT, GEM and Abr under the same doses for 48h. Whole cell extract was prepared for Western Blotting.

Statistical analysis was determined by one-way ANOVA. Our result showed that combining PMT with single treatment of GEM or Abr, or double treatment of GEM plus GEM significantly reduced pSTAT3 levels. GEM induced significant increase of survivin, which can be significantly suppressed by combining with PMT. The combination PMT plus Abr significantly reduced survivin levels as compared to the standard Abr and GEM. Since both pSTAT3 and survivin are involved in therapeutic resistance of GEM and Abr, this data indicates that PMT has the potential as combination agent to reduce resistance to GEM and Abr through downregulation of pSTAT3 and survivin levels.

Major goal 5: Determine the therapeutic activity of PMT with GEM plus Abraxane in preclinical model

Obtained IACUC approval, ACURO document under preparation

- **What opportunities for training and professional development has the project provided?**
  - Through actively participating in Cancer Biology Journal Club and seminars at UT Health San Antonio, I have kept up with breakthroughs in cancer research.
  - **Technical skills:** This year's training has provided outstanding opportunities to learn new techniques, such as CyTOF and generating stable KD cell lines. I have also gained expertise in preclinical models such as orthotopic implantation and dissection in mouse model through participating in other ongoing projects. My collaborative work has resulted in 2 publications (1 under review) during the year 2020.
  - **Presentation skills:** I have improved my presentation skills by presenting journal articles during lab meetings and at Cancer Biology Journal Club here at UT Health San Antonio (UTHSA), and presenting my exciting findings at lab meetings, committee meetings, student seminars and at the UTHSA Mays Cancer Center (MCC) Retreat.
  - **Scientific writing skills:** I have improved my scientific writing skills through submitting abstract to UT Health San Antonio MCC retreat and working as a teaching assistant (TA) in the Scientific Writing class.
  - **Mentoring skills:** I have developed my mentoring skills by working as a TA in the Scientific Writing class and by mentoring undergraduate students, rotation students, medical students and research assistants in Dr. Kumar's lab.
  - **Critical thinking skills:** Through weekly one-on-one meetings with my mentor Dr. Kumar, I have greatly improved my critical thinking

skills and my understanding of the big pictures in cancer research and therapy.

- **Skills for experimental design:** Through trainings in graduate school, I have learned to conduct experiments with scientific rigor – to design experiments with positive and negative controls, to perform experiments in biological and technical replicates, and to analyze data with appropriate statistical methods.

- **How were the results disseminated to communities of interest?**

  - I have disseminated my research findings at the UTHSA Mays Cancer Center (MCC) Retreat and UT Health San Antonio student seminars, to clinicians and researchers who may or may not be aware of the role of pancreatic microenvironment and its role in the resistance to chemotherapy.

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

  - During Year 2 I plan to (i) initiate and complete the experiments proposed in Major task 1, subtask 2-3 to investigate glutamine (Q)-mediated communication between pancreatic stellate cells (PSC) and pancreatic cancer cells (PCC); (ii) continue working on experiments proposed in Major Task 2, subtask 1 to test the effect of palmtine (PMT) on Q-release from PSCs; (iii) initiate and complete preclinical studies to determine the efficacy of the combination treatment PMT, gemcitabine (GEM) and abraxane (Abr); (iv) I also plan to compile my current data regarding the roles of PMT to suppress Q-induced cancer hallmarks in PCCs for a manuscript for publication.

4. **IMPACT:**

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

  - <u>Impact on the base of knowledge of pancreatic cancer:</u> The pancreatic cancer microenvironment plays a vital role in the development and chemoresistance of pancreatic cancer. Pancreatic stellate cells (PSCs) has been known for their prominent role in the fibrotic stromal response to reduce drug perfusion and contribute to chemoresistance. However, their communication with pancreatic cancer cells (PCCs) through secreting factors has not been thoroughly elucidated. Our findings added to the knowledge that PSCs stimulate cancer growth through secreting glutamine.

  - <u>Impact on pancreatic cancer drug development:</u> our result showed that palmatine (PMT), through (i) inhibiting glutamine release from PSCs, (ii) inhibiting glutamine induced proliferation in PCCs and (ii)

through potentiating conventional chemotherapy agents GEM and Abr has the potential to be developed as a combinational agent for pancreatic cancer patients.

- **What was the impact on other disciplines?**
  - Since PMT targets STAT3 signaling – a upregulated signaling that contributes to therapeutic resistance in pancreatic cancer – developing PMT as a combinational agent will help clinicians select treatment strategies based on individual patient's gene expression profile.
  - Persistent activation of STAT3 signaling is frequently detected in other cancer tumor types (including breast cancer, lung cancer, prostate cancer, ovarian cancer etc.). If our results show that PMT is a specific inhibitor of STAT3 signaling, our findings can be expanded to other tumor types beyond pancreas.
- **What was the impact on technology transfer**
  - We have submitted a patent on palmatine before the start of this project, that has the potential to lead to the initiation of a start-up company, or to be used in the government of industry.
- **What was the impact on society beyond science and technology?**
  - We have identified potential novel therapeutic regimen (PMT in combination with GEM or Abr) using cell culture models. We expect to determine the efficacy of this combination using appropriate preclinical models through experiments proposed as part of goals in Y2. If successful these studies can lead to development of a clinical trial testing the identified combination in pancreatic cancer patients. This will have major impact in reducing the pancreatic cancer burden in the society.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
  - 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**
  - Nothing to Report
- **Significant changes in use or care of human subjects, vertebrate animals, 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.**

    Presentations:

    8-23-2019 **UT Health San Antonio (UTHSA) Graduate School of Biomedical Sciences (GSBS), Cancer Biology Journal Club**

    Topic: EGFR-Pak Signaling Selectively Regulates Glutamine Deprivation-Induced Macropinocytosis

    8-29-2019 **Lab Meeting Presentation - Journal presentation**

    Topic: Nutritical cues regulate pancreatic tumor's "cell drinking"

    10-10-2019 **Lab Meeting Presentation - Journal presentation**

    Topic: Proteomic analysis of ECM during pancreatic ductal adenocarcinoma progression reveal different contributions by tumor and stromal cells

    11-06-2019 **UTHSA Mays Cancer Center Retreat**

    Topic: Targeting glutamine addiction in pancreatic cancer

    11-22-2019 **UTHSA GSBS - Graduate student Committee Meeting**

    Topic: Novel role of glutamine in the tumor-stromal interaction of pancreatic ductal adenocarcinoma

    5-7-2020 **Lab Meeting Presentation**

    Topic: Research Progress Update

5-13-2020 **UTHSA GSBS - Graduate student Committee Meeting**

Topic: Novel role of glutamine in the tumor-stromal interaction of pancreatic ductal adenocarcinoma

5-29-2020 **UTHSA GSBS Cancer Biology Discipline - Student seminar presentation**

Topic: Novel role of glutamine in the tumor-stromal interaction of pancreatic ductal adenocarcinoma

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

  Nothing to Report

- **Technologies or techniques**
  - Nothing to Report
- **Inventions, patent applications, and/or licenses**
  - We have submitted a patent on palmatine before the start of this project
- **Other Products**
  - Nothing to Report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

  PI: Xiaoyu Yang (no change)

- **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?**
  - Nothing to Report

8. **Special Reporting Requirements**

- Nothing to Report

9. Appendices

Award Chart

W81XWH-19-1-0596 (CA181275) : Glutamine-Mediated Tumor-Stromal Interaction: A Novel Target for Pancreatic Cancer Treatment

PI: Xiaoyu Yang, UT Health San Antonio, TX **Budget:** $228,750.00

Topic Area: FY19 Peer Reviewed Cancer Research Program **Mechanism:** Horizon Award

Research Area(s): 0804 **Award Status: 08/15/2019 – 08/14/2021**

Study Goals:

The goal of this project is to investigate the role of glutamine in the communication between pancreatic stellate cells and pancreatic cancer cells to promote pancreatic cancer progression and to clarify how palmatine can disrupt this communication to potentiate response to conventional therapeutics

Specific Aims:

Specific aim 1: To determine the mechanism through which glutamine mediates pancreatic stellate cells - pancreatic cancer cells communication to promote cancer survival hallmarks (proliferation, invasion, metastasis and cell death) and the ability of palmatine to inhibit this process.
Specific aim 2: To determine the effectiveness of palmatine-mediated glutamine inhibition to improve response to conventional therapeutic agents.

Key Accomplishments and Outcomes:

Publications: none to date
Patents: none to date
Funding Obtained: none to date