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14. ABSTRACT

This study investigated a function for cell surface GRP78 in regulating prostate cancer stem-like cells. In year 1, we showed that cell surface GRP78 was upregulated in prostate cancer sphere cultures compared to that in cells grown in 2D tissue culture plates, indicating enrichment of cell surface GRP78+ cancer stem like cells in sphere culture. We also showed that the signaling axis activated by cell surface GRP78 is upregulated in prostate cancer cells grown in sphere culture compared those grown in adherent culture. In year 2, we showed that prostate cancer cells are heterogeneous, being composed of cell surface GRP78(+) and GRP78(-) tumor cells. By cell sorting we showed that the GRP78(+) prostate cancer cells, but not the GRP78(-) cells, exhibited cancer stem-like cell behavior. Furthermore an GRP78 monoclonal antibody inhibited sphere forming ability of GRP78(+) prostate cancer cells. In year 3, we demonstrated that GRP78(+) cells, relative to GRP78(-) cells, express increased phospho-Akt , increased phospho-GSK-3, and increased Snail-1. Surprisingly, the majority of phospho-Akt was localized in the nucleus of cell surface GRP78(+) tumor cells. Finally, we tested the hypothesis that GRP78(+) tumor cells exhibit increased tumor initiating activity relative to GRP78(-) tumor cells. These studies are still in progress, but initial results indicate that GRP78(-) tumor cells are more tumorigenic than GRP78(+) tumor cells. We postulate that the GRP78(-) tumor cells restored GRP78 expression in vivo in order to survive, and will test this hypothesis by harvesting tumors from animals. Collectively, our studies indicate that GRP78 drives cancer stem-like cell growth, and can be targeted with a GRP78 antibody. Future studies will investigate whether these antibodies suppress prostate cancer growth in animal models.

15. SUBJECT TERMS

prostate cancer, cell surface GRP78, cancer stem cell, Snail-1

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PROGRESS REPORT:

1. **INTRODUCTION:** This study investigated a function for cell surface GRP78 in regulating prostate cancer stem-like cells. In year 1, we optimized sphere culture conditions for studying prostate cancer stem-like cell growth in vitro. In addition, we showed that cell surface GRP78 was upregulated in prostate cancer sphere cultures compared to that in prostate cancer cells grown in 2D tissue culture plates, indicating enrichment of cell surface GRP78+ cancer stem like cells in sphere culture. We also showed that the signaling axis activated by cell surface GRP78[1] is upregulated in prostate cancer cells grown in sphere culture compared those grown in adherent culture. In year 2, we produced data indicating that prostate cancer cells are heterogeneous, being composed of cell surface GRP78(+) and GRP78(-) tumor cells. By cell sorting we showed that the GRP78(+) prostate cancer cells, but not the GRP78(-) cells, exhibited cancer stem-like cell behavior (i.e. demonstrated sphere forming ability in non-adherent conditions in the absence of serum). Furthermore, an antibody that binds to the carboxyl terminal domain of GRP78 inhibited sphere forming ability of GRP78(+) prostate cancer cells. In year 3, we studied whether signaling associated with cell surface GRP78 (Akt/GSK-3/Snail-1) was upregulated in GRP78(+) relative to GRP78(-) prostate cancer cells. Our results in this period demonstrated that GRP78(+) cells, relative to GRP78(-) cells, express increased phospho-Akt , increased phospho-GSK-3, and increased Snail-1. These studies showed that cell surface GRP78(+) prostate cancer stem-like cells support an Akt/GSK-3/Snail-1 signaling axis. In our previous studies demonstrating that cell surface GRP78 activates Akt in prostate cancer cells, we assumed that activated Akt was located in the cytosol. Surprisingly, by separating nuclear from cytosolic proteins, we observed that the majority of phospho-Akt was localized in the nucleus of cell surface GRP78(+) tumor cells. Importantly, Akt could not be detected in the nucleus of GPR78(-) prostate cancer cells. We were unable to initiate animal studies testing tumor initiating activity of GRP78-sorted prostate cancer cells in year 3 because Dr. Chi, our animal study co-investigator, lost his animal technician. Accordingly, we obtained a one-year, no cost extension, allowing us to pursue animal studies in 2016. These studies tested the hypothesis that GRP78(+) tumor cells exhibit increased tumor initiating activity relative to GRP78(-) tumor cells. These studies are still in progress as of the date of this report, but initial results indicate that GRP78(-) tumor cells are actually more tumorigenic than the GRP78(+) tumor cells. We postulate that the GRP78(-) tumor cells restore GRP78 expression in order to survive, and will test this hypothesis in January of 2017 by harvesting tumors from injected animals. Collectively, our studies indicate that GRP78 drives cancer stem-like cell growth, and can be targeted with monoclonal antibodies directed against the carboxyl terminus of GRP78. Future studies are planned to investigate efficacy of these monoclonal antibodies in blocking prostate cancer growth in animal models.

2. KEYWORDS: GRP78, prostate cancer stem-like cell, prostaspheres

3. OVERALL PROJECT SUMMARY (Tasks refer to those outlined in approved Statement of Work):

Task 1: Establish adherent and prostasphere cultures from prostate cancer cell lines.

During the first year of this award, we optimized conditions for growing prostasphere cultures from human prostate cancer cell lines (1LN and DU145). We found that addition of methylcellulose to sphere cultures was necessary to prevent non-specific cell-cell aggregation (See Fig. 1A). We also investigated the effect of seeding density on sphere formation, and determined that a seeding density of 12,000 cells/well of a 6 well plate generated the optimal sphere density for counting (Fig. 1A and 1C). These conditions were optimal for establishing prostasphere cultures for both human prostate cancer cell lines studied (1LN and DU145) (Fig. 1A and 1C). We also showed our ability to grow secondary spheres from dissociated primary spheres (Fig. 1B), an assay reflecting the self renewing activity of cancer stem-like cells. Collectively, these results show that we have optimized cell culture conditions for addressing a role for cell surface GRP78 in regulating prostate cancer stem-like cells (remaining tasks in Statement of Work).

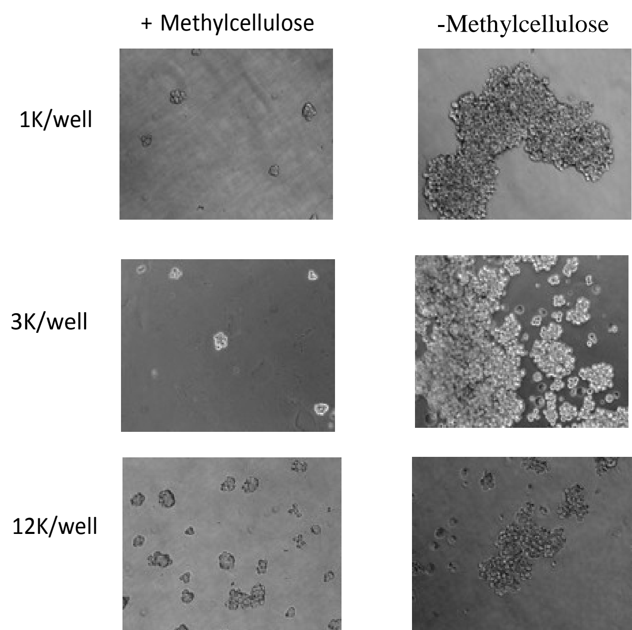


Fig. 1A: Optimizing sphere cultures for prostate cancer cells. 1LN cells form distinguishable spheres in media containing methylcellulose, but not in media lacking methylcellulose. 1-LN cells were seeded into wells of a 6 well tissue culture dish at the indicated seeding densities in sphere medium (RPMI1640 containing 4 µg/ml human insulin, 20 ng/ml EGF, 20 ng/ml FGF, 100 units/ml penicillin and 100 µg/ml streptomycin) either containing methylcellulose or lacking methylcellulose (1%, 400 cp). Pictures (100 X) were taken on day 4.

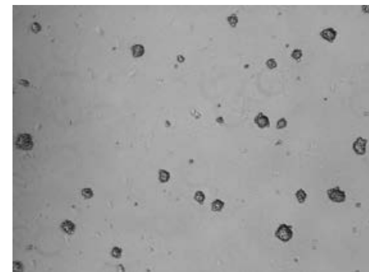
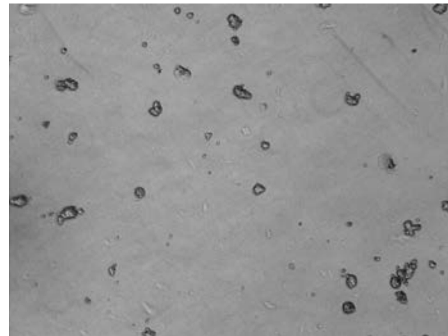


Fig. 1B: Primary 1LN spheres, when dissociated and reseeded into sphere culture form secondary spheres, indicative of self-renewing activity. Primary 1LN prostaspheres were harvested, washed with PBS and dissociated using Trypsin/EDTA
Cells were put through a 40um cell strainer and re-plated in sphere medium in 6-well plates, as in Fig. 1A.

Fig. 1C: Development of sphere culture conditions for DU145 prostate cancer cells. DU145 cells were seeded at 12,000 cells per well of 6 well plate using methylcellulose-containing sphere medium, as described in Fig. 1A. Pictures (100X) were taken on d4.



Task 2: Investigate the relative expression of cell surface GRP78 in adherent prostate cancer cells and in prostate cancer stem-like cells.

Using the prostasphere culture conditions optimized in Fig. 1, we next investigated cell surface GRP78 expression in prostasphere-derived tumor cells and in prostate cancer cell lines. As shown in Fig. 2A, GRP78 expression levels were increased in prostasphere culture compared to 2D culture conditions. ***These studies demonstrate that cell surface GRP78 levels are increased in prostate cancer stem-like cells (prostaspheres) relative to that in bulk adherent prostate cancer cells (achieving goal for Tasks 1 and 2, Statement of Work).***

Upon performing these experiments, we recognized that the level of cell surface GRP78 on prostate cancer cells is too low to permit GRP78 sorting, as was originally planned in Task 6 (Statement of Work). Based on a previous report indicating that chemotherapy treatment enriches for cancer stem-like cells[2], we next investigated the hypothesis that chemotherapy treatment of human prostate cancer cells increases GRP78 cell surface expression. As shown in Fig. 2B, chemotherapy treatment of DU145 cells for 4 days significantly increased the percent cell surface GRP78-positive cells, raising the possibility that chemotherapy enriches for prostate cancer stem-like cells that express cell surface GRP78.

We next tested whether these chemo-residual cells exhibit increased sphere forming ability compared to untreated cells. Data in Fig. 2C demonstrate that sphere forming ability of chemo-residual DU145 cells was not increased relative to that of untreated DU145 cells, indicating that chemotherapy does not enrich for prostate cancer stem-like cells. Accordingly, we attempted cell surface GRP78 sorting on untreated DU145 cells (see Task 6).

Fig. 2A: Flow cytometry for cell surface Grp78 on 1LN sphere cells. 1LN cells were harvested with 2mM EDTA from adherent tissue culture plates. 1-LN sphere cultures were established as in Fig. 1A. After 5 days, spheres were harvested with 2mM EDTA in HBSS. Cells were stained (1 ug/10^5 cells) with anti-Grp78 antibodies (N-20) from Santa Cruz. Control is goat IgG.

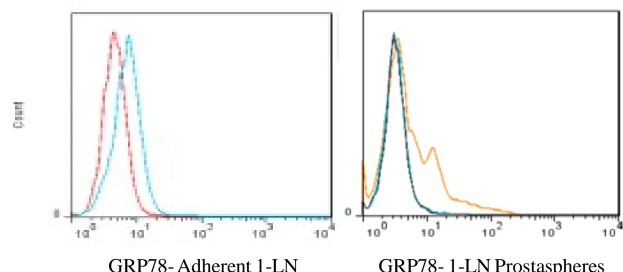
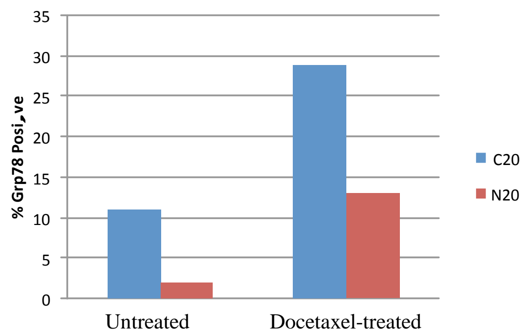
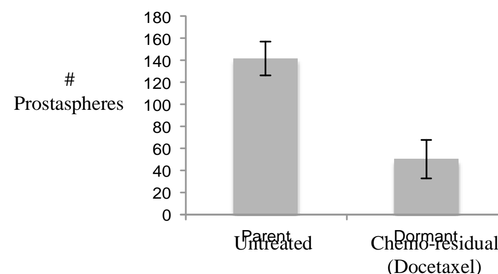


Fig. 2B and C: Chemotherapy treatment enriches for prostate cancer cells with increased GRP78 cell surface expression, but not increased sphere forming ability. DU145 cells were incubated with 10 nM docetaxel for 4 days. Media was removed, cells were washed with PBS and fresh media was added. Cells were incubated for an additional 6 days, then harvested. **B.** Cells were stained for Grp78 using C-terminal (C-20) or N-terminal (N-20) GRP78 antibody. % cells positive for cell surface GRP78 are indicated. **C.** Sphere forming ability of untreated and chemo-residual cells was then assessed. Prostaspheres were generated in serum-free RPMI 1640 (Gibco®, Life Technologies, USA) supplemented with B27, EGF (20 ng/ml) and FGF (20 ng/ml). Prostasphere media included 1% methylcellulose to prevent sphere aggregation. Untreated and docetaxel-treated DU145 were harvested, counted and seeded in triplicate on ultra-low attachment 6-well plates (Corning Inc., Corning, NY, USA) at equal densities (DU145 = 10,000 cells/well; SUM159 = 25,000 cells/well). After 12 d of sphere growth, spheres >50 µm were counted on the GelCount™ Colony Counter (Oxford Optronix, Abington, UK). GelCount™ sphere settings were optimized for each cell line and were not changed between trials. Results represent three independent trials. Error bars represent the standard error of the mean (SEM).

B.

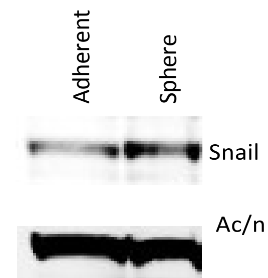


C.



Task 4: Investigate the relative expression of Snail-1, a GSK-3 target, in adherent prostate cancer cells and in prostate cancer stem-like cells.
To begin to address the hypothesis that Snail-1 expression is driven by cell surface GRP78 expression in prostate cancer stem-like cells, we determined Snail-1 protein levels in 1LN prostate tumor cells grown in 2D culture, as well as in the same cells grown as prostaspheres (using the methods described in Fig.1). As shown in Fig. 3, Snail-1 expression was significantly increased in 1LN cells cultured as prostaspheres compared to the levels observed in 1LN cells grown in monolayer culture. These findings show that increased cell surface GRP78 expression in prosaspheres (compared to adherent prostate tumor cells) is associated with increased expression of Snail-1. ***This work addresses the goal of Task 4 in the Statement of Work (testing the hypothesis that Snail-1 expression levels are increased in prostate cancer stem-like cells relative to that in bulk adherent prostate cancer cells).***

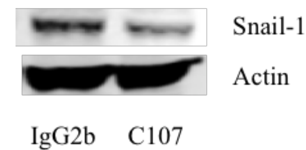
Fig. 3: Snail-1 expression is increased in 1LN cells grown in sphere culture compared to that in 1LN cells grown as monolayer. Total cellular proteins were extracted from 1LN 2D culture (Adherent) and from 1LN grown in sphere culture as in Fig. 1A (Sphere). Equivalent amounts of protein were subjected to SDS-PAGE and probed with a Snail-1 antibody (Cell Signaling) or Actin antibody (Sigma), followed by IRdye-conjugated secondary antibody. Proteins were detected by Odyssey infrared imaging.



Task 5: Determine the effect of incubating human prostate cancer stem-like cells with monoclonal antibodies specific for N-terminal and C-terminal domains of cell surface GRP78 on Akt/GSK-3/Snail-1 signaling.

To directly address the hypothesis that cell surface GRP78 drives Snail-1 expression in human prostate cancer cells, we incubated 1LN cells with a C-terminal GRP78-neutralizing monoclonal antibody (or isotype control antibody). After 24 hours, total cellular proteins were extracted, and Snail-1 levels were assessed by immunoblotting. As shown in Fig. 4, this GRP78 neutralizing antibody significantly reduced Snail-1 protein levels, supporting the hypothesis that cell surface GRP78 drives Snail-1 expression in prostate tumor cells.

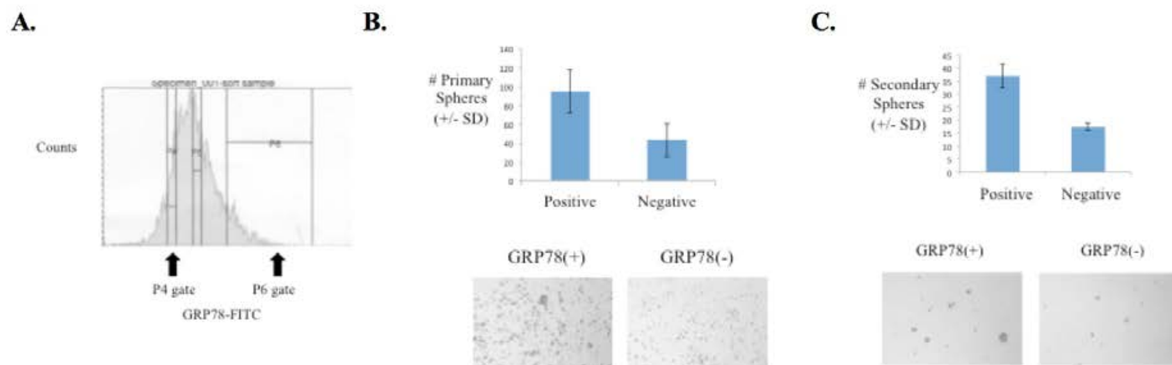
Fig. 4: Snail-1 expression is reduced in prostate cancer cells incubated with a C-terminal GRP78-specific monoclonal antibody. 1-LN cells were incubated for 24 h with a C-terminal GRP78 antibody (C107) or isotype control antibody (IgG2b) at 1µg/mL. Equivalent amounts of total cellular protein were immunoblotted with Snail-1 or actin antibodies, as described for Fig. 3.



Task 6: Investigate the relative ability of cell surface GRP78(+) and cell surface GRP78(-) prostate cancer cells to grow as self-renewing prostaspheres in vitro.

We performed a sorting strategy for isolating cell surface GRP78(+) and cell surface GRP78(-) prostate cancer cells (DU145) with the goal of testing the relative abilities of these two populations to grow as self-renewing spheres, a property of cancer stem-like cells. We showed that GRP78(+) tumor cells were more efficient in sphere formation than GRP78(-) tumor cells (Fig. 5B). We next sought to determine if cell surface GRP78(+) tumor cells exhibited self-renewing activity by harvesting primary spheres and reseeding these cells into a secondary sphere assay. As shown in Fig. 5C, cell surface GRP78(+) tumor cells were more efficient at primary and secondary sphere formation than GRP78(-) tumor cells, indicating that the former exhibited self-renewing activity.

Figure 5: Measuring self-renewing activity of cell surface GRP78(+) and cell surface GRP78(-) DU145 prostate cancer cells. DU145 cells were harvested with 2 mM EDTA and stained with anti-GRP78-alexa fluor 488 (10 ug/10^6 cells) and 7AAD to exclude dead cells. **A.** Cells were sorted into GRP78-positive (P6 gate) and GRP78-negative (P4 gate) populations**. B.** Sorted populations were grown as prostaspheres. Prostasphere number from three wells (+/- SEM) was determined on d8 (top panel). Pictures of representative wells are shown (bottom panel). **C.** Primary prostaspheres were dissociated with trypsin and seeded at equal numbers into secondary sphere assays. Prostasphere numbers from three wells were



Task 3: Determine relative activities of Akt and its substrate GSK-3 in adherent prostate cancer cells and in prostate cancer stem-like cells.

Having shown by cell sorting that cell surface GRP78(+) but not cell surface GRP78(-) prostate cancer cells can grow as self-renewing spheres (Fig. 5), we next sought to address the hypothesis that cell surface GRP78-stimulated Akt signaling drives this activity. Accordingly, we extracted nuclear and cytosolic proteins from sorted GRP78(+) and GRP78(-) prostate cancer cells, and performed immunoblotting to measure levels of phospho-Akt and Akt. Classical Akt signaling occurs in the cytosol. Thus we were surprised to observe that the predominant phospho-Akt signal in GRP78(+) tumor cells was localized in the nucleus. As demonstrated in Fig. 6, cell GRP78(+) cells exhibited a 100-fold increase in nuclear phospho-Akt levels compared to that in GRP78(-) cells (as determined by densitometry). In contrast, cytosolic phospho-Akt levels were equal in GRP78(+) and GRP78(-) cells. These studies are the first to show an association between cell surface GRP78 expression in prostate cancer cells and nuclear Akt signaling. We also performed immunoblotting for GSK-3, a direct substrate of Akt. As shown in Fig. 3, GRP78(+) prostate cancer cells exhibited a 2.4 fold increase in phospho-GSK-3 levels compared to that in GRP78(-) cells. Interestingly, phosphorylated GSK-3 protein in cell surface GRP78(+) tumor cells was localized in the nucleus. GSK-3 exists as two isoforms (alpha and beta). As shown in Fig. 6, cell surface GRP78 expression was associated with increased phospho-GSK-3 alpha, but not increased phospho-GSK-3 beta. We also investigated levels of Snail-1, a downstream target of GSK-3, in these GRP78 sorted populations. As shown in Fig. 7, cell surface GRP78(+) tumor cells exhibited increased levels of nuclear Snail-1.

Figure 6: Cell surface GRP78 expression in prostate cancer cells is associated with activation of a nuclear Akt/GSK-3 signaling axis. Cell surface GRP78(+) and (-) DU145 prostate cancer cells were obtained by cell sorting, as in Fig. 5A. Nuclear and cytosolic proteins were extracted using our previously published methods. Equivalent amounts of protein were subjected to SDS-PAGE, and immunoblotted with phospho-Akt, Akt, phospho-GSK-3, GSK-3, GAPDH, and Lamin A antibodies, followed by IRdye conjugated secondary antibody.

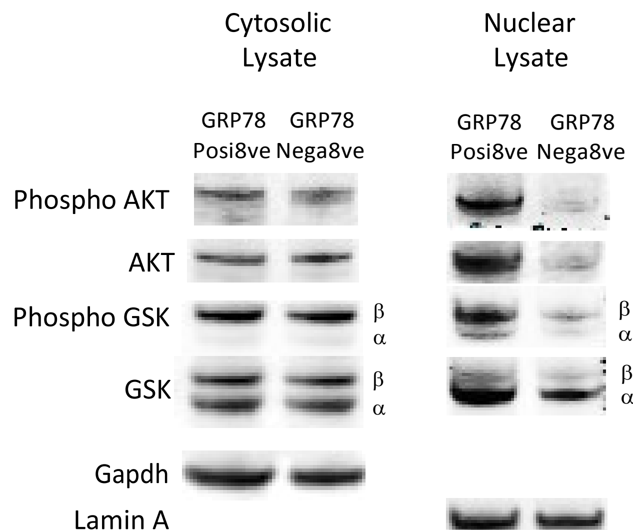
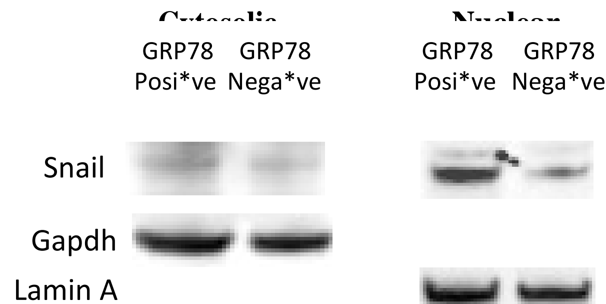


Figure 7: Cell surface GRP78 expression in prostate cancer cells is associated with increased levels of nuclear Snail-1, a transcription factor associated with cancer stemness. Cell surface GRP78(+) and (-) DU145 prostate cancer cells were obtained by cell sorting, as in Fig. 5A. Nuclear and cytosolic proteins were extracted using our previously published methods. Equivalent amounts of protein were subjected to SDS-PAGE, and immunoblotted with Snail-1, GAPDH, and Lamin A antibodies, followed by IRdye conjugated secondary antibody. Protein bands were detected using a Licor Odyssey Fluorescent scanner.



Task 7: Using Snail-1 shRNAs, assess the importance of Snail-1 for cell surface GRP78 regulation of the growth of self-renewing prostate cancer stem-like cells. Our attempts to make prostate cancer cell transfectants expressing Snail-1 shRNAs failed because we discovered that Snail-1 is essential for the survival of the prostate cancer cell lines. In order to address our hypothesis that Snail-1 is required for GRP78 regulation of prostate cancer stem-like cell growth, it will be necessary to generate conditional knockout cells using Snail-1 shRNA constructs whose expression is controlled by an inducible promoter. These experiments will require generation of inducible shRNA constructs, which could not be constructed in the time frame of this grant budget.

Task 8: Determine the relative ability of cell surface GRP78(+) and cell surface GRP78(-) prostate cancer cells to initate tumor growth in a xenograft model.
An important behavior of cancer stem-like cells is their enhanced tumor initiating activity relative to cancer cells lacking stem behaviors. Thus, we next sought to determine the relative abilities of sorted cell surface GRP78(+) and (-) prostate cancer cells to establish tumors in a xenograft model.

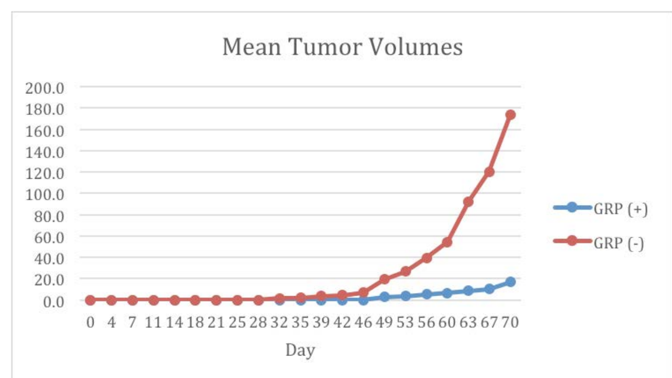


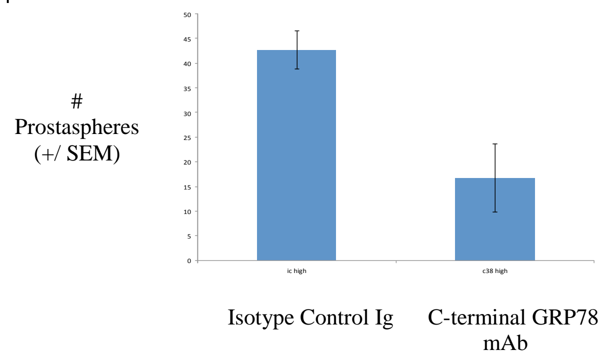
Figure 8: GRP78(-) prostate cancer cells exhibit increased tumor initiating activity relative to GRP78(+) tumor cells. Mice (12 per group) were injected in the mammary fat pad with 100 tumor cells. Tumor volume was assessed twice weekly. Note that tumors will be isolated from these animals in 2017 to address the hypothesis that GRP78(-) tumor cells acquired cell surface GRP78 expression in vivo as a means of tumor cell survival.

Task 9c: Test effects of administering GRP78 antibody to mice on tumor initiating activity of prostate cancer stem-like cells (grown in prostasphere culture). Initiation of this study was reliant on results obtained from studies described for Task 8, investigating the relative tumor initiating activity of GRP78(+) and GRP78(-) tumor cells isolated from prostate cancer cell lines by cell sorting. Results from Task 8 disproved our hypothesis. Specifically, we did not observe an ability of cell surface GRP78(+) tumor cells to initate tumors in vivo more efficiently tan cell surface GRP78(-) tumor cells. To attempt to understand results obtained from this study ,we are testing the hypothesis that GRP78(-) sorted tumors evolved compensatory pathways to initiate tumor growth in vivo. We are testing this hypothesis by measuring GRP78, Snail-1, and phospho-Akt expression in tumors isolated from these animals. Studies of these compensatory pathways are taking longer than the original grantduration. In summary, we did not initiate the in vivo studies described in Task 9c because relevant hypotheses in the grant (i.e. in Task 8) were not proven due to unpredicted complications with tumor cell adaption *in vivo*.

Task 9b: Determine the effect of incubating prostate cancer stem-like cells with GRP78 monoclonal antibodies on their ability to grow as self-renewing cancer stem-like cells.

Based on our successful demonstration that cell surface GRP78(+) DU145 cells exhibit increased sphere forming ability compared to cell surface GRP78(-) cells (see Fig. 5), we investigated the effect of a C-terminal GRP78 monoclonal antibody on sphere forming ability of cell surface GRP78(+) DU145 cells. As shown in Fig. 4, this C-terminal antibody prevented sphere growth of cell surface GRP78(+) tumor cells.

Figure 9: C-terminal GRP78 monoclonal antibody (C38) suppresses the ability of cell surface GRP78(+) DU145 tumor cells to grow as self-renewing prostaspheres. DU145 prostate cancer cells were sorted into cell surface GRP78(+) and cell surface GRP78(-) tumor cell populations as described in Fig. 5A. Sorted populations were then cultured as prostaspheres (using the methods in Fig. 1) +/- C-terminal GRP78 monoclonal antibody C38 or an isotype contr antibody (all antibodies at 5 µg/mL). Spheres were counted on d8, using the methods described in Fig. 1.



Task 10: Using a xenograft model, examine the effect of an antibody specific for a C-terminal domain of cell surface GRP78 on human prostate cancer growth. As discussed for Aim 9c, initiating studies for this Task required proving our hypothesis in Task 8. Specifically, in Task 8, we did not show that GRP78(+) tumor cells were more efficient than GRP78(-) tumor cells in establishing tumors in vivo. We are currently testing whether compensatory pathways are upregulated in GRP78(-) tumors isolated in vivo to explain this result. These unexpected complications prevented us from initiating studies investigating ability of GRP78 antibodies to inhibit tumor growth in vivo.

4. KEY RESEARCH ACCOMPLISHMENTS:

- Determined that cell surface GRP78 expression levels are increased in prostasphere culture compared to levels in adherent culture, suggesting that GRP78 levels are increased in prostate cancer stem-like cells.

- Determined that Snail-1 expression is elevated in prostaspheres relative to adherent prostate cancer cells.

- Obtained evidence that incubation of prostate cancer cells with a C-terminal GRP78 antibody reduces Snail-1 expression levels.

- Developed a strategy for sorting cell surface GRP78(+) and cell surface GRP78(-) prostate cancer cells.

- Showed that cell surface GRP78(+) prostate cancer cells are more efficient at prostasphere formation than cell surface GRP78(-) prostate cancer cells.

- Demonstrated efficacy of a C-terminal GRP78 monoclonal antibody (C38) in targeting prostate cancer stem-like cells.

- Showed that cell surface GRP78(+) prostate cancer cells are more efficient at prostasphere formation and self renewal than cell surface GRP78(-) prostate cancer cells.

- Demonstrated that cell surface GRP78 expression is associated with the activation of an Akt/GSK-3 signaling axis.

- Provided the first evidence that cell surface GRP78(+) prostate cancer cells support ***nuclear*** Akt signaling.

- Showed that Snail-1, a transcription factor associated with stemness and regulated by Akt/GSK-3 signaling, is increased in GRP78(+) tumor cells relative to GRP78(-) tumor cells.

- Demonstrated that tumor initiating activity of cell surface GRP78(-) tumor cells is REDUCED relative to that of cell surface GRP78(+) tumor cells. ***Ongoing studies will address the hypothesis that GRP78(-) tumor cells injected into these mice upregulate cell surface GRP78 as a means of surviving in vivo.***

5. CONCLUSIONS: By performing cell sorting on DU145 prostate cancer cells, we showed that cell surface GRP78(+) prostate cancer cells exhibit increased ability to grow as self-renewing prostaspheres [compared to cell surface GRP78(-) prostate cancer cells]. We also demonstrated that cell surface GRP78(+) tumor cells exhibit increased Akt activity, and that this activity is localized to the nucleus. Cell surface GRP78(+) tumor cells have increased phospho-GSK-3 levels, as well as increased levels of the nuclear transcription factor Snail-1, an established determinant of stemness. Studies from this past year indicate that cell surface GRP78(+) prostate cancer cells exhibit reduced tumor initiating activity in immunodeficient mice relative to cell surface GRP78(-) tumor cells. In January of 2017, we will address the hypothesis that tumor cells from mice injected with cell surface GRP78(-) tumor cells acquire cell surface GRP78 expression as a means of survival. Results from these studies will be important to future work investigating whether targeting cell surface GRP78 on prostate cancer cells with a monoclonal antibody inhibits prostate cancer growth/metastasis.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Peer-reviewed scientific journals:

1. S. Li, M. Kennedy, S. Payne, K. Kennedy, V. L. Seewaldt, S. V. Pizzo, R. E. Bachelder, Model of tumor dormancy/recurrence after short-term chemotherapy. *PLoS One* **9**, e98021 (2014)10.1371/journal.pone.0098021).

Presentations:

2012 Duke University Medical Center, Prostate Cancer Research Forum "Targeting Prostate Cancer Stem-like cells through cell surface-expressed GRP78"

2013 Duke University School of Medicine, Basic Science Research Day. "Size matters: Targeting giant tumor cells to prevent recurrence"

2013 Duke University Medical Center, Department of Pathology Grand Rounds. "Targeting chemotherapy-enriched dormant tumor cells to prevent recurrence"

7. INVENTIONS, PATENTS, AND LICENSES: none to report

8. REPORTABLE OUTCOMES:

- Developed a model of prostate cancer dormancy/recurrence after short-term chemotherapy
- Demonstrated that cell surface GRP78(+) tumor cells exhibit increased cancer stem-like behaviors

- Showed that GRP78 monoclonal antibodies targeting the carboxyl-terminal domain inhibit prostate cancer stem-like growth

9. OTHER ACHIEVEMENTS: none to report

10. REFERENCES:

1. Misra S, Toole BP, Ghatak S. Hyaluronan constitutively regulates activation of multiple receptor tyrosine kinases in epithelial and carcinoma cells. J Biol Chem. 2006;281(46):34936-41. doi: 10.1074/jbc.C600138200. PubMed PMID: 16959784.
2. Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, et al. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst. 2008;100(9):672-9. doi: 10.1093/jnci/djn123. PubMed PMID: 18445819.