Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009-12/31/2010

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant ME Number or SAP Number:** 4100047631

6. **Project Number and Title of Research Project:** *6 - Role of O-GlcNac Transferase as a Biomarker and Therapeutic Target for Prostate Cancer*

7. **Start and End Date of Research Project:** 1/1/2009 – 6/30/2010

8. **Name of Principal Investigator for the Research Project:** Mauricio Reginato, PhD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 112,500

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Reginato	Assistant Professor	5%	$ 5468
Garcia	Professor	3%	$ 6427
Vosseller	Assistant Professor	5%	$ 5468
Lynch	Graduate student	50%	$14218
Lupica	Research Assistant	10%	$ 5585

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
O-GlcNac Transferase: Novel Therapeutic Target for Prostate Cancer	☐NIH ■ Other federal (specify:_ DOD-Prostate Cancer Research Program _) ☐Nonfederal source (specify:_____)	June 2009	$450,000	$0
O- GlcNAc Transferase: A Novel Therapeutic Target for Prostate Cancer Metastasis	☐NIH ☐ Other federal (specify:_____) ■ Nonfederal source (specify: Prostate Cancer Foundation)	Jan 2011	$300,000	Not reviewed yet

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____X____ No_____

If yes, please describe your plans:

Will re-apply for DOD PCRP in 2011 and incorporate this data into ACS grant in 2011.

12. Future of Research Project. What are the future plans for this research project?

Finish Aim 1 (immunohistochemical analysis) and identify specific prostate cancer targets of O-GlcNAcation. Determine mechanism of how OGT regulates FoxM1 degradation. Test novel whether inhibiting OGT blocks prostate cancer metastasis in vivo.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes__x_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female				
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian				
Other				
Unknown				
Total			**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____x_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___x_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

We have, for the first time at DUCOM, created prostate cancer tissue microarrays. These will be useful for other researchers interested in examining expression of proteins as it related to prostate cancer.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____x_____ No_____

If yes, please describe the collaborations:

We have begun a collaboration with Glaxo-SmithKline Pharmaceuticals in Collegeville, PA. They will be screening their chemical libraries for inhibitors of OGT. We will then test them in our in vitro and in vivo cancer models.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____x____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____x_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Based on our preliminary data, we hypothesize that increased level and activity of OGT occurs during prostate cancer progression and contributes to prostate cancer tumor growth through modulation of the transcription factor FoxM1. This proposal is directed towards addressing this novel hypothesis.

Aim 1. Analyze whether increase of OGT expression and O-GlcNAc modifications occurs at a significant frequency in human prostate cancers *in vivo* and to determine at what stage of tumorigenesis it occurs.
Aim 2. Characterize role of OGT in prostate cancer cell proliferation.
Aim 3. Determine mechanism of how OGT regulates cell cycle regulator FoxM1.

<u>Summary of Progress.</u>
<u>Aim 1. Analyze whether increase of OGT expression and O-GlcNAc modifications occurs at a significant frequency in human prostate cancers *in vivo* and to determine at what stage of tumorigenesis it occurs.</u>

Our preliminary data showed that OGT and levels of O-GlcNAc levels were elevated between one normal immortalized prostate cell line compared to one prostate cancer cell line. We examined a number of other prostate cell lines and found that nontumorigenic prostate cells RWPE-1 and NPTX-1532 contain low levels of O-GlcNAc and OGT compared to PC3, PC3-ML, and DU145 prostate cancer cell lines (Fig. 1F). This data is consistent with Onconime data from four different studies showing that OGT is elevated at RNA level in prostate carcinoma compared to normal or benign prostate tissue (Fig. 1A-D).

With collaboration with Dr. Garcia, we were able to create one prostate cancer TMA containing 45 cases of human prostate carcinoma. However, we determined that OGT antibody we were using for IHC was picking up non-specific proteins since prostate cancer cells in which we knocked down OGT were still staining positive for OGT using IHC (we had verified knockdown in these same cells via western analysis). Thus, we are currently testing new OGT antibodies that are more specific for OGT and work well in our positive controls before staining our prostate

cancer TMAs. In addition, we have started staining prostate cancer tissue with total O-GlcNAc antibody to determine if we can detect increases between normal and prostate cancer tissue.

Aim 2. Characterize role of OGT in prostate cancer cell proliferation.

To investigate the role of increased *O*-GlcNAc modifications in prostate cancer, we reduced the levels of OGT in PC3-ML cells by using two different shRNA constructs delivered via lentiviral infection (Fig. 2A). After reducing OGT expression, these cells were then subjected to 3D culture for eight days (Fig. 2B), after which the cells were harvested and counted. As shown in Figure 2C, targeting OGT significantly ($p < 0.05$) reduced growth in 3D by approximately 60%; overall acini size was reduced as well (Fig. 2B). In addition to reducing growth and 3D morphogenesis, targeting OGT in PC3-ML cells reduces anchorage-independent colony formation in soft agar assays (Fig. 2D). This approximate 80% reduction in anchorage-independent growth was statistically significant ($p < 0.05$) compared to control shRNA constructs (Fig. 2E). While targeting OGT in PC3-ML cells reduces malignant growth in 3D, targeting OGT in the RWPE-1 cell line (Fig. 3A) does not alter growth (Fig. 3B) or 3D morphogenesis (Fig. 3C).

To determine the role of reducing OGT in prostate cancer cell growth we examined expression of Ki-67, a marker for cell proliferation. Reducing OGT expression in prostate cancer cells PC3-ML, but not normal RWPE-1 cells, decreased expression of KI-67 by 3 fold (Fig. 4A). Decrease in the population of cells expressing Ki-67 suggested that cell cycle regulators may be altered by reducing OGT expression. Knockdown of OGT results in a significant induction of $p27^{Kip1}$ levels in PC3-ML cells (Fig. 4B). Degradation of $p27^{Kip1}$ is primarily regulated by the SCF^{SKP2} E3 ubiquitin ligase complex (Chu *et al.*, 2008). This complex includes the F-Box protein Skp2 that targets CDK inhibitors for degradation during G1/S transition. We find that OGT knockdown in PC3-ML cells (data not shown) decreases Skp2 expression. One level of Skp2 regulation is through transcriptional activation by FoxM1 (Wang *et al.*, 2005). We find in PC3-ML cells reducing OGT expression leads to significant decreases in FoxM1 protein levels (Fig. 4B).

Targeting OGT reduces invasive phenotypes in PC3-ML cells via decreasing MMP-2 and -9 expression.

When subjected to 3D culture conditions, the PC3-ML cell line exhibits a morphological phenotype that is consistent with invasive potential (Fig. 1C). By targeting OGT with shRNA in PC3-ML cells, 3D growth appears more organized and similar to that of the RWPE-1 cell line (Fig. 1C), compared to control shRNA constructs (Fig 5A). This reduction in invasive morphology upon decreased OGT expression led us to assay the invasive potential of PC3-ML cells in transwell invasion chambers. As shown in Figure 5B, there is a an approximate 60% reduction in the ability of PC3-ML cells to invade through the Matrigel coating and transwell membrane with decreased OGT expression. Since it has previously been established that matrix metalloproteinase-2 and -9 (MMP-2 and -9) are associated with prostate cancer progression and metastasis [8, 9], we examined MMP-2 and-9 mRNA levels in cells in which OGT expression was reduced via shRNA. By knocking down OGT, we observe an approximate 70% reduction in MMP-2 and approximate 50% reduction in MMP-9 mRNA levels by QRT-PCR (Fig. 5C). Additionally, targeting OGT reduces levels of MMP-2 protein in PC3-ML cells (Fig. 5D). Both MMP-2 and MMP-9 have recently been shown to be transcription targets of FoxM1 (ref).

Targeting OGT Reduces Angiogenic Potential and VEGF Expression in PC3-ML Cells.

FoxM1 has been to shown to regulate VEGF expression in breast, pancreatic, and gastric cancers (Li, Cancer Research 2009), suggesting that decreasing OGT may also inhibit VEGF expression and angiogenic potential of prostate cancer cells. We first determined if reducing levels of OGT in PC3-ML cells altered endothelial tube formation, an indirect measure of angiogenesis. After 24 hrs of stably expressing control or OGT shRNA in PC3-ML we collected conditioned media. This conditioned media was then used to stimulate human umbilical vein cells (HUVEC) in an endothelial tube formation assay; HUVECs were seeded onto a bed of Matrigel and then covered with PC3-ML cell conditioned media. After 6 hours, the HUVECs were imaged and tube length quantified using iVision software. As seen in Figure 6A, there is a statistically significant ($p < 0.05$, *t*-test) reduction in the ability of conditioned media from PC3-ML cells expressing OGT shRNA to stimulate tube formation in HUVECs as compared to media from control shRNA cells. RNA was harvested from the same PC3-ML cells used to condition media and levels of VEGF RNA were determined. As expected, levels of OGT RNA were decreased in those cells expressing OGT shRNA, moreover, VEGF RNA levels were also significantly ($p < 0.05$, *t*-test) decreased by 50-60 percent (Fig. 6B), consistent with the decreased ability of OGT knock-down cells to stimulate endothelial tube formation (Fig. 6A). We also examined expression of VEGF protein expression and found significant decrease in VEGF levels in prostate cancer cell expressing OGT shRNA (Fig. 6C). Thus, we show for the first time that reducing OGT in prostate cancer cell blocks angiogenic potential of cancer cells which correlates with decrease of VEGF RNA and protein levels.

Pharmacological inhibition of OGT reduces malignant growth and invasion of prostate cancer cells.

Previously identified inhibitor of OGT catalytic activity (Gross, et al) ST060266, as well as novel second generation inhibitors (SW8, and SW9) provided by collaborator Dr. Suzanne Walker (Harvard Medical School), were used to reduce global *O*-GlcNAcation in PC3-ML cells (Fig. 7A). Cells were subjected to 3D culture and treated with OGT inhibitors. Both SW8 and SW9 treatment showed reduced growth of PC3-ML cells compared to control or ST060266 compound (Fig. 7B). Additionally, a reduction in anchorage-independent growth was also seen in soft agar assays as a result of OGT inhibitor treatment (Fig. 7C). Novel SW9 OGT inhibitor treatment of PC3-ML cells also reduced invasive potential in transwell invasion assays by approximately 80% (Fig. 7D).

Thus, taken together these results suggest that reducing O-GlcNacation in prostate cancer cells potently and selectively blocks cancer cell growth which is associated with elevated p27 levels and decrease in FoxM1 levels. Moreover, reducing O-GlcNAc levels blocks prostate cancer cell invasion and angiogenesis. These results suggest that targeting OGT may be a viable therapeutic target in treating aggressive prostate cancers and possible block metastasis.

<u>Aim 3. Determine mechanism of how OGT regulates cell cycle regulator FoxM1.</u>

OGT knockdown leads to a post-transcriptional decrease in FoxM1 levels. Protein levels of exogenous wildtype FoxM1 were still decreased following OGT knockdown. However, expression levels of FoxM1 mutant missing N-terminal destruction (D) and KEN box motif was not inhibited by knockdown of OGT (Caldwell, et. al., *Oncogene, in press*). Since these degradation motifs regulate proteasomal degradation of FoxM1, we examined whether OGT knockdown increases proteasomal degradation of endogenous FoxM1. To test this directly, we

treated OGT knockdown cells with proteasome inhibitor lactacystin for 6 hours. Reduction of FoxM1 levels in OGT knockdown cells was blocked by treatment with lactacystin (data not shown) suggesting that OGT regulation of FoxM1 protein levels is mediated by the proteasome. Reduction of FoxM1 protein is not a part of a global alteration in protein degradation, as protein levels of other transcription factors regulated via the proteasome were not altered in OGT depleted cells (Caldwell, et al., *in press*).

Since FoxO1, a member of the Fox transcription family, has been recently shown to be directly O-GlcNAcated (18), we have carefully examined whether FoxM1 is directly O-GlcNAcated. We were unable to detect any level of O-GlcNAcation of FoxM1 overexpressed in MDA-MB-231 cells following FoxM1 immunoprecipitation (IP). However, under similar conditions endogenous Sp1, a transcription factor known to be modified by O-GlcNAc (15), was highly O-GlcNAcated (Caldwell, et al., *in press*). Moreover, our collaborator Keith Vosseller, Ph.D. (Drexel University College of Medicine) and expert in O-GlcNAc mass spectometry was unable to detect any amino acid sites on FoxM1 that were O-GlcNAcated following mass spectrometry analysis of FoxM1 protein (data not shown). Thus, our data suggest that decreasing O-GlcNAc in cancer cells leads to proteasomal degradation of FoxM1 dependent on KEN & D box motifs but independent of direct O-GlcNAcation of FoxM1. Future studies will examine mechanism of how OGT regulates FoxM1 degradation.

Figure 1. OGT levels and O-GlcNAc modifications are elevated in prostate cancer. (a) (Dhanasekaran, Barrette et al. 2001), *(b)* (Yu, Landsittel et al. 2004), **(c)** (Dhanasekaran, Dash et al. 2005), *(d)* (Varambally, Yu et al. 2005), and *(e)* (Wallace, Prueitt et al. 2008) microarray data available through the *Oncomine* database re-analyzed to compare expression of OGT in normal prostate tissue and prostate carcinoma. *(f)* Normal prostate cell lines RWPE-1 and NPTX-1532 and prostate cancer cell lines PC3, PC3-ML and DU145 were lysed and subjected to immunoblot with the indicated antibodies. *(g)* Brightfield and confocal micrographs comparing organized RWPE-1 and invasive PC3-ML growth in three-dimensional culture.

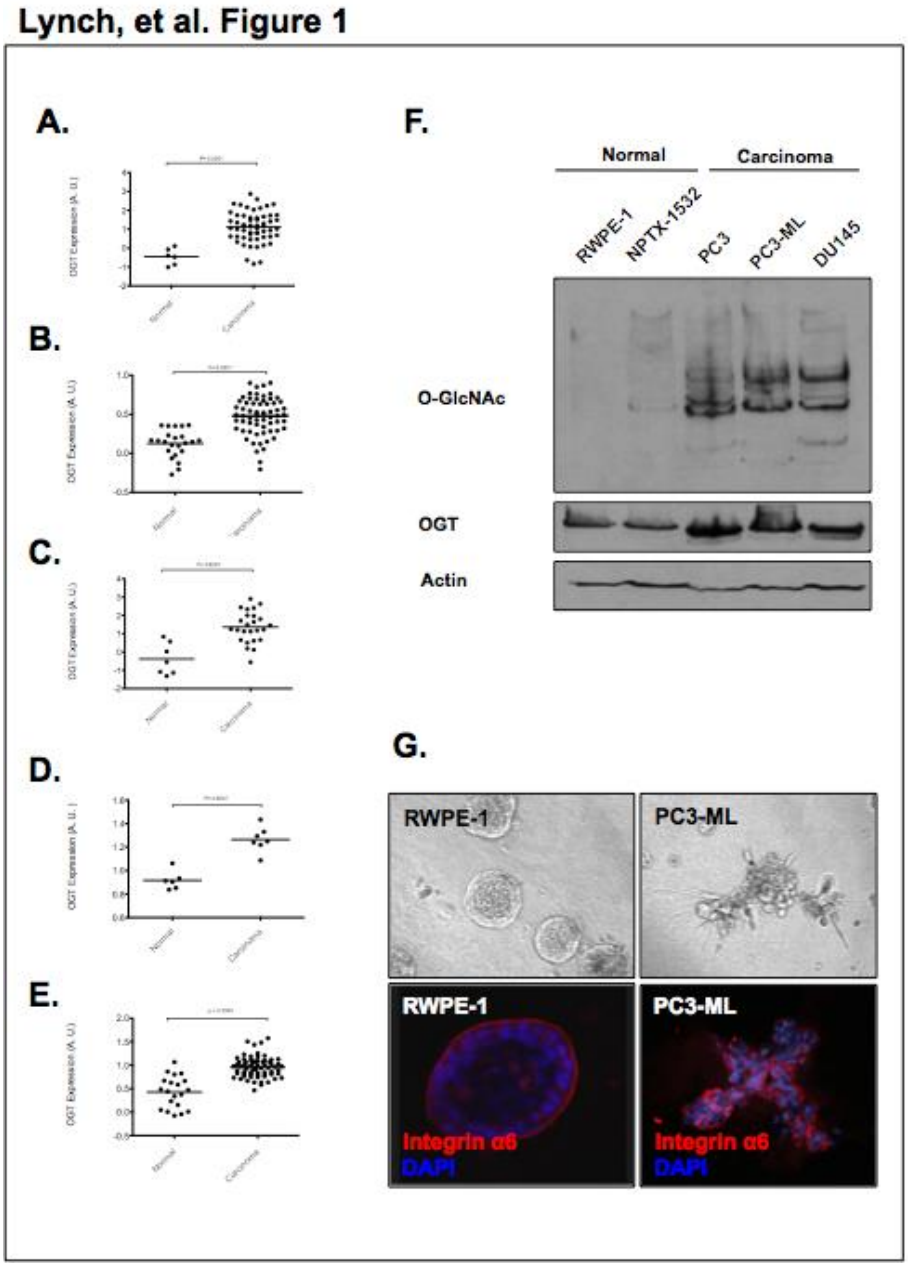
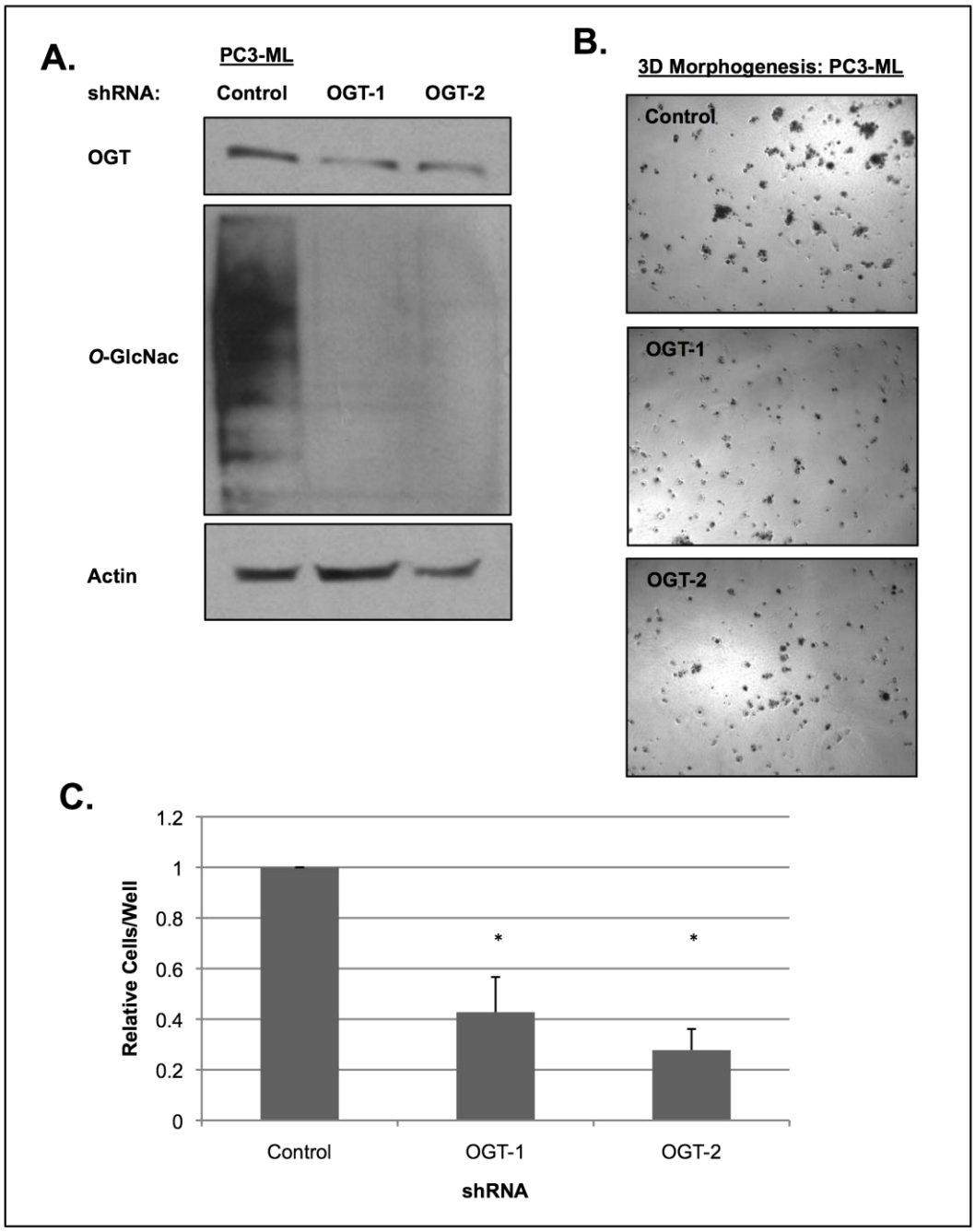


Figure 2. Targeting OGT decreases growth in vitro. (a) PC3-ML cells were infected with either Control (scramble), OGT-1 or OGT-2 shRNA-containing lentivirus, lysed and immunoblotted with the indicated antibodies. Control and OGT knock-down PC3-ML cells were grown in three-dimensional culture for 8 days *(b)*, harvested and quantified *(c)*. Growth in three-dimensional culture is significantly reduced (t-test, $p < 0.05$); data is expressed as average ($n = 3$) cells per well, relative to control. *(d)* PC3-ML cells stably expressing OGT shRNA were subjected to soft agar colony formation assays over 16 days. Representative wells stained with p-iodonitrotetrazolium-violet. *(e)* Targeting OGT results in a statistically significant (t-test, $p < 0.05$) reduction in anchorage-independent growth compared to control. Data expressed as average ($n = 3$) colony formation, relative to control. * indicates $p < 0.05$.

Lynch, et al. Figure 2



Lynch, et al. Figure 2

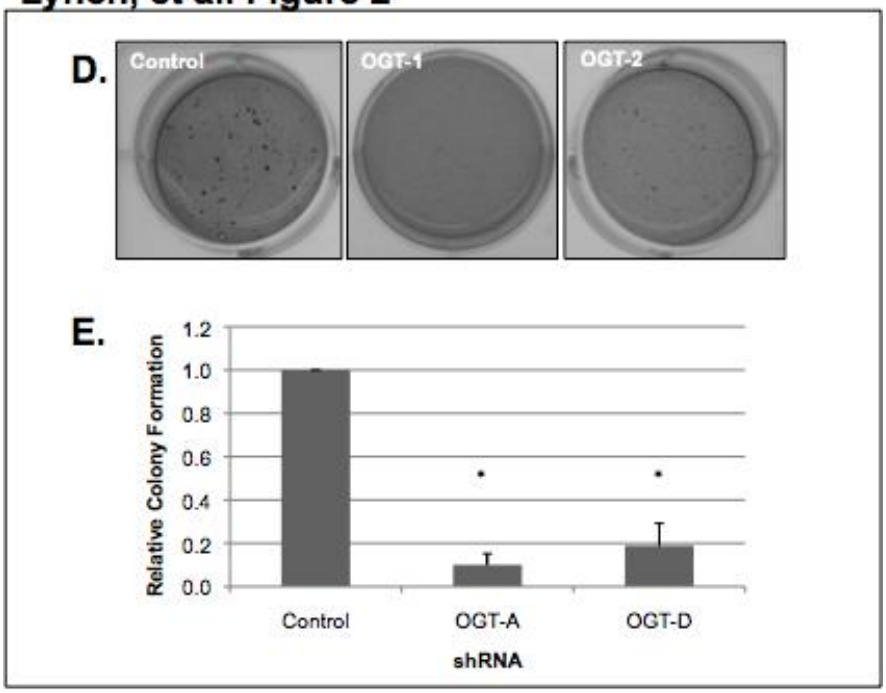


Figure 3. Reducing OGT expression has no effect on normal immortalized prostate epithelial cells. *(a)* RWPE-1 cells were infected with either Control (scramble), OGT-1 or OGT-2 shRNA-containing lentivirus, lysed and immunoblotted with the indicated antibodies. Control and OGT knockdown RWPE-1 cells were grown in three-dimensional culture for 8 days *(b)*, harvested and quantified *(c)*. Growth in three-dimensional culture was not significantly reduced (t-test, $p < 0.05$); data is expressed as average ($n = 3$) cells per well, relative to control.

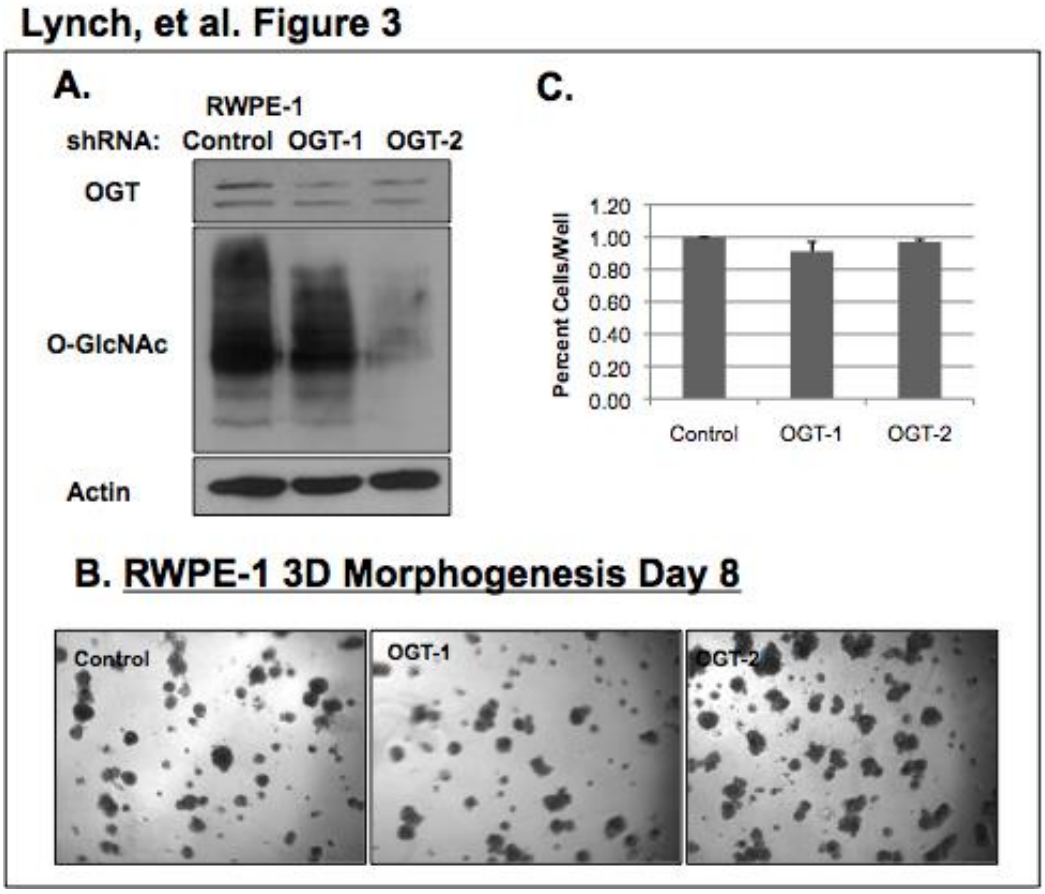


Figure 4. Targeting OGT reduces cell proliferation in prostate cancer cells. (a) RWPE-1 and PC3-ML cells control and OGT knock-down cells were stained for Ki-67 and DAPI, then counted. *(b)* Whole-cell lysate from PC3-ML cells infected with control, OGT-1 or OGT-2 shRNA were resolved with SDS-PAGE and immunoblotted with indicated antibodies.

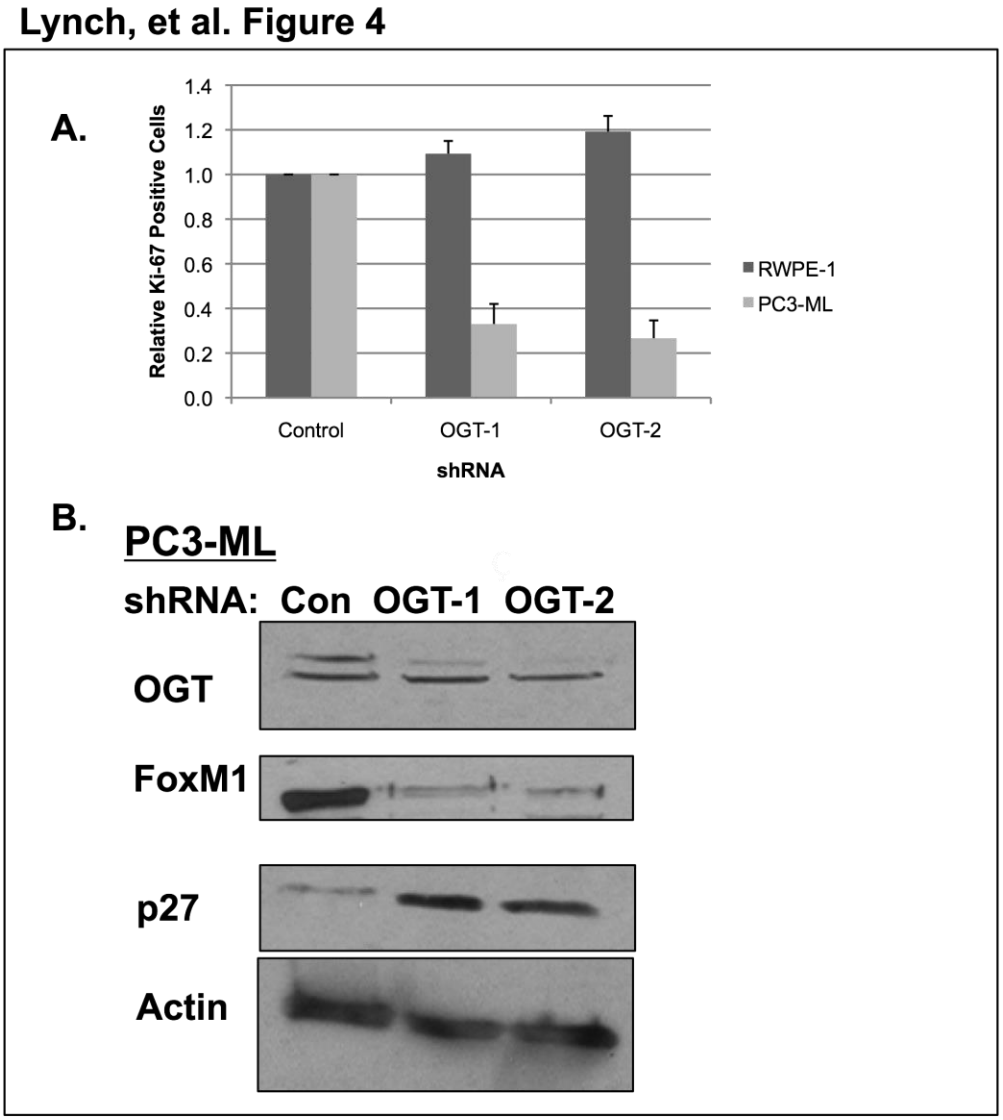


Figure 5. Targeting OGT reduces invasive phenotypes through MMP-2, -9 reduction. (a) PC3-ML cells infected with control, OGT-1 or OGT-2 shRNA and subjected to three-dimensional culture for 12 days. Cells were then stained as indicated for confocal microscopy. *(b)* PC3-ML cells infected with control, OGT-1 or OGT-2 shRNA were seeded in transwell invasion chambers with a serum gradient to induce chemotaxis for 24 hours, at which time invading cells were stained with DAPI and counted. Data are presented as average (n =3) invasion relative to control. *(c)* Whole-cell lysate from PC3-ML cells infected with control, OGT-1 or OGT-2 shRNA were resolved with SDS-PAGE and immunoblotted with OGT, MMP-2 and actin antibodies. *(d)* Total RNA from PC3-ML cells infected with control, OGT-1 or OGT-2 shRNA were analyzed to QRT-PCR for expression of OGT, MMP-2 and -9, normalized to cyclophilin A. Data are presented as average (n = 3) expression relative to control. * indicates $p < 0.05$.

Lynch, et al. Figure 5

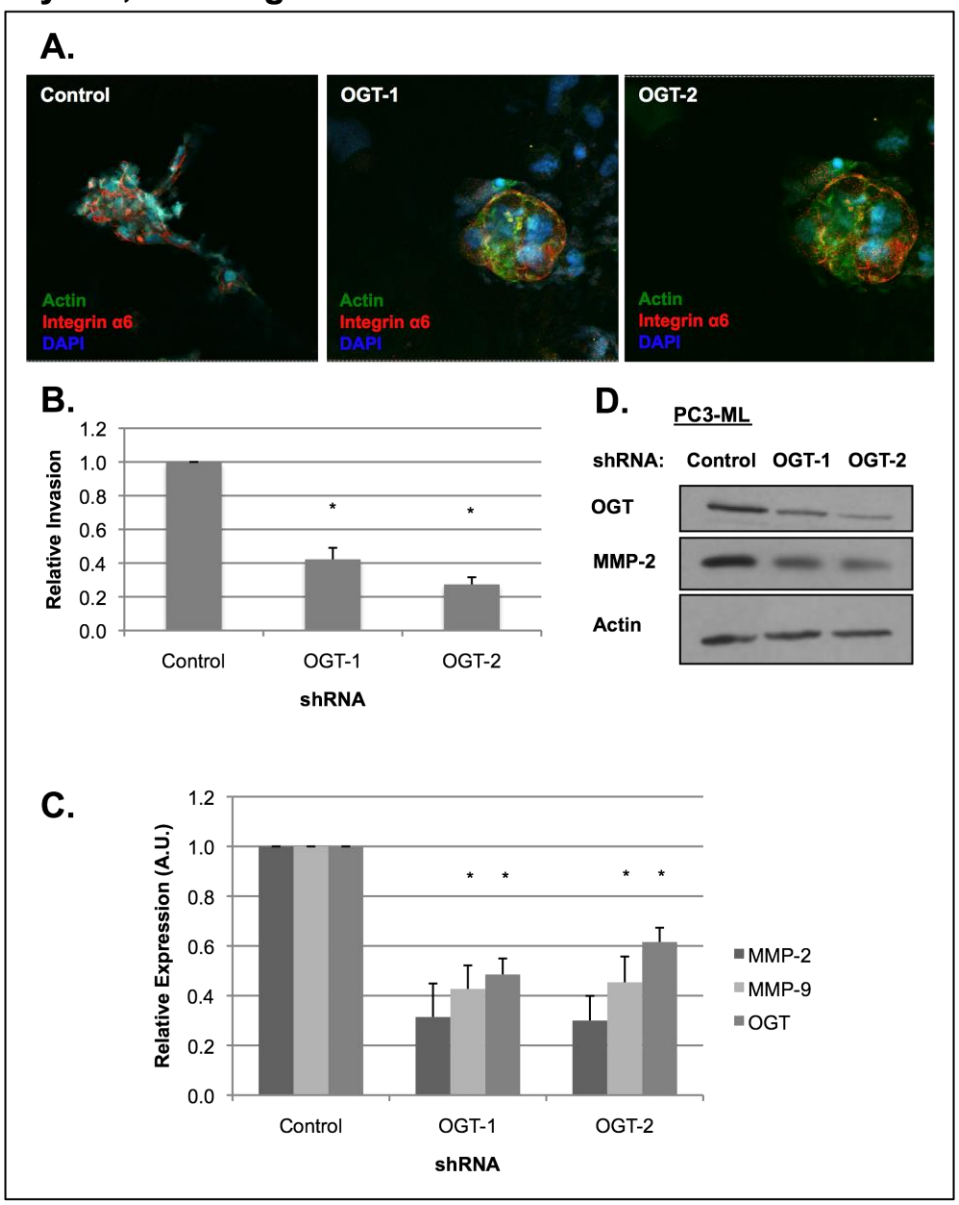


Figure 6. Targeting OGT decreases PC3-ML angiogenic potential in vitro. **A.** Conditioned RPMI 1640 media from control, OGT-1 or OGT-2 shRNA expressing PC3-ML cells was used to overlay HUVECs seeded on a bed of Matigel for 6 hours in endothelial cell tube formation assays. Relative tube length, compared to control shRNA was calculated; error bars indicate SEM (n = 3). *p > 0.05. Representative images are depicted above (5X magnification). **B.** Total RNA was collected from PC3-ML cells expressing control, OGT-1 or OGT-2 shRNA and was subjected to QPCR for VEGF and OGT. Data were normalized to cyclophilin A and are presented as relative expression to control shRNA; error bars indicate SEM (n = 3). *p > 0.05. **C.** Immunoblotting with the indicated antibodies for PC3-ML cells expressing control, OGT-1 or OGT-2 shRNA.

Lynch, et al. Figure 6

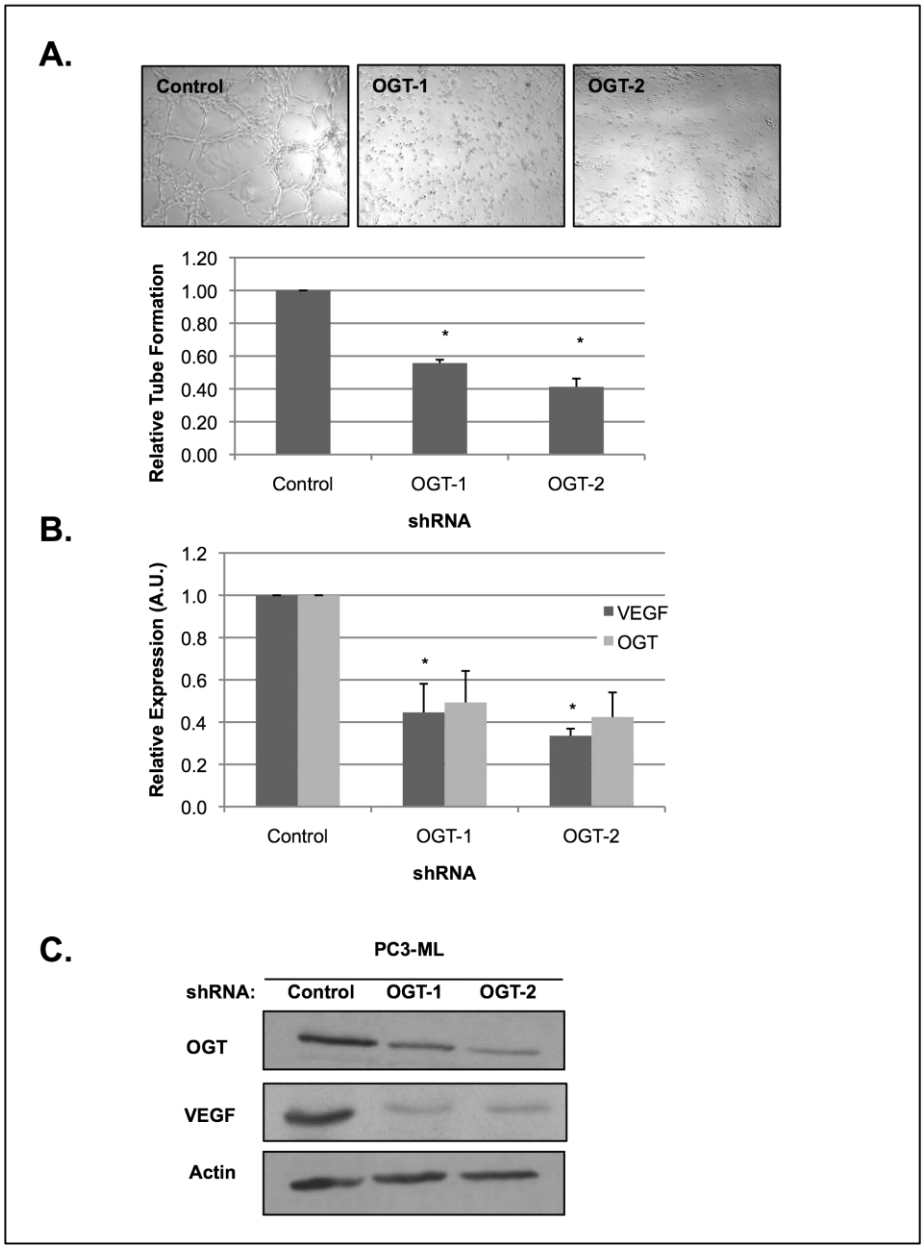
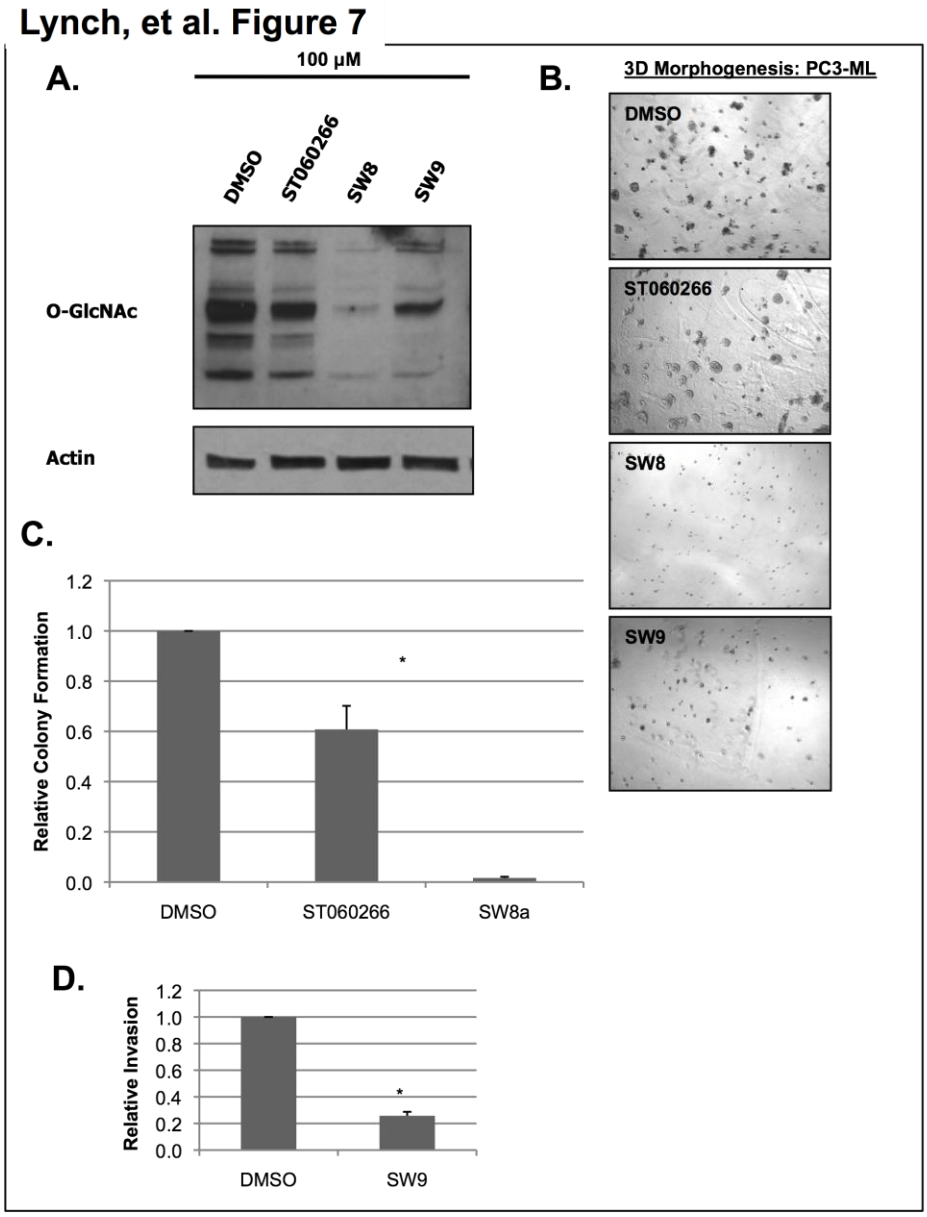


Figure 7. Pharmacological inhibition of OGT activity reduces malignant phenotypes in PC3-ML cells. **(a)** PC3-ML cells were treated with either 100 μM control (DMSO) or OGT inhibitors ST060266, SW8 and SW9. Cells were lysed and immunoblotted with the indicated antibodies. **(b)** During three-dimensional culture, PC3-ML cells were treated with either 100 μM control (DMSO) or OGT inhibitors ST060266, SW8 and SW9 over 8 days. **(c)** PC3-ML cells were seeded in transwell invasion chambers, along with 100 μM control (DMSO) or OGT inhibitor (SW9). After 24 hours, invading cells were stained with DAPI and counted. Data are presented as average (n = 3) invading cells, relative to control. **(d)** PC3-ML cells were assayed for colony formation in the presence of 100 μM control (DMSO) or OGT inhibitors (ST060266 or SW8a) over 27 days. At which time, colonies were stained with p-iodonitrotetrazolium-violet and counted. Data are expressed as average (n = 3) colony formation, relative to control. * indicates $p < 0.05$.



References

Dhanasekaran, S. M., T. R. Barrette, et al. (2001). "Delineation of prognostic biomarkers in prostate cancer." Nature **412**(6849): 822-826.

Dhanasekaran, S. M., A. Dash, et al. (2005). "Molecular profiling of human prostate tissues: insights into gene expression patterns of prostate development during puberty." FASEB J **19**(2): 243-245.

Varambally, S., J. Yu, et al. (2005). "Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression." Cancer Cell **8**(5): 393-406.

Wallace, T. A., R. L. Prueitt, et al. (2008). "Tumor immunobiological differences in prostate cancer between African-American and European-American men." Cancer Res **68**(3): 927-936.

Yu, Y. P., D. Landsittel, et al. (2004). "Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy." J Clin Oncol **22**(14): 2790-2799.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
____x_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should

be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___x_____ No_____

If yes, please describe your plans:

Have a manuscript in preparation that will be submitted in one month entitled:

Lynch, T., Jackson, S., Shahriari, K., Walker, S., Vosseller, K., and Reginato, M. O-GlcNAc Transferase is Required for Prostate Cancer Growth, Invasion and Angiogenesis. (in preparation)

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its

impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The work performed under this award has, for the first time, identified OGT as potential therapeutic target for treating prostate cancer. We have begun to understand molecular pathway involved in how OGT contributes to cancer phenotypes via regulation of oncogenic transcription factor FoxM1. Future studies will address whether OGT inhibitors block prostate cancer using animal models and identify molecular pathway of OGT regulation of FoxM1 degradation.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

We have for the first time tested novel OGT inhibitors (SW8 and SW9) as anti-cancer agents. These compounds have profound anti-growth and invasion effects in prostate cancer cells. They will be further tested using additional in vitro and in vivo assays in future studies.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

NAME **Mauricio J. Reginato**	POSITION TITLE **Assistant Professor of Biochemistry and Molecular Biology**
eRA COMMONS USER NAME **reginato1**	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(If applicable)*	YEAR(s)	FIELD OF STUDY
Penn State University	B.S.	1989	Biology/Physiology
University of Pennsylvania	Ph.D.	1998	Pharmacology
Harvard Medical School	Postdoctoral	2004	Cancer Cell Biology

POSITIONS AND EMPLOYMENT:

6/89-8/92	**Associate Scientist**, Department of Investigative Toxicology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA
9/92-5/98	**Graduate Student**, Laboratory of Dr. Mitchell A. Lazar, Department of Pharmacology, University of Pennsylvania, Philadelphia, PA
5/98-7/04	**Postdoctoral Fellow**, Laboratory of Dr. Joan S. Brugge, Department of Cell Biology, Harvard Medical School, Boston, MA
8/04-	Assistant Professor**, Co-Director Imaging Facility, Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA**

Other Experience and Professional Memberships

2006-present	Grant Reviewer, New Jersey Commission on Cancer Research
2009	Ad-hoc Grant Reviewer, Ireland Health Research Board
2004-present	Ad-hoc reviewer, Genes & Dev., Oncogene, Mol. Bio. Cell, J. Biol. Chem., Breast Cancer Research
2010	Grant Reviewer, Department of Defense Breast Cancer Research Program
2010	AACR 2011 Annual Meeting Program Committee: Member of the Cellular Stress Responses Subcommittee

Honors/Award

1991: Impact Award-Smithkline Beecham Pharmaceuticals
1992: Fontaine Fellowship Predoctoral Award-University of Pennsylvania
1992: Predoctoral Fellowsip Award-NIGMS
1995: Predoctoral Minority Award-Merck-Dupont Pharmaceuticals
1998: Saul Weingrad Best Dissertation Award: Pharmacological Sciences, University of Pennsylvania
1999: Susan G. Komen Breast Cancer Foundation Postdoctoral Fellowship
2010: Professional Enrichment and Growth Award, Drexel University College of Medicine
2010: Young Investigator Award, Drexel University College of Medicine

C. Selected Peer-reviewed Publications (Selected from 23 peer-reviewed publications)

Most relevant to the current application

1. Caldwell, S. A., Jackson, S. R., Shahriari, K. S., Lynch, T., Sethi, G., Walker, S., Vosseller, K., and **Reginato, M. J.** (2010) Nutrient Sensor O-GlcNAc Transferase Regulates Breast Cancer Tumorigenesis via Targeting of the Oncogenic Transcription Factor FoxM1. Oncogene, May 13; 29:2831-42. PMCID: 20190804
2. Whelan, K. A., Caldwell, S. A., Shahriari, K., Jones, L., Johannes, G. and **Reginato, M. J.** (2010) Hypoxia suppression of Bim and Bmf blocks anoikis and luminal clearing during mammary morphogenesis. Mol. Biol. Cell, 21: 3829-3837. PMID: 20861305
3. Haenssen, K. K., Caldwell, S. A., Shahriari, K., Jackson, R., Whelan, K., Klein-Szanto, A., and **Reginato, M. J.** (2010) ErbB2 requires integrin α5 for anoikis resistance via c-Src regulation of receptor activity in human mammary epithelial cells. J. of Cell Science, Apr 15; 123:1373-82. PMCID: 20332114
4. Palacios, C., **Reginato, M. J.** and López-Rivas A. (2010) Cellular $FLIP_L$ plays a survival role and regulates morphogenesis in breast epithelial cells. Biochim Biophys Acta. Oct 13. [Epub ahead of print]
5. Sodunke, T. R., Turner, K. K., Caldwell, S. A., McBride, K. W., **Reginato, M. J.** and Noh, N. (2007) Micropatterns of Matrigel for Three-Dimensional Epithelial Cultures. Biomaterials 28:4006-4016. PMCID: 17574663

Additional recent publications of importance (in chronological order)

1. Katz, D., **Reginato, M. J.,** and Lazar, M. A. (1995) Functional regulation of thyroid hormone receptor variant TRa2 by phosphorylation. Mol. Cell. Biol. 15, 2341-2348. PMCID: 7739517
2. **Reginato, M. J.,** Zhang, J., and Lazar M. A. (1996) DNA-independent and DNA-dependent mechanisms regulate the differential heterodimerization of the isoforms of the thyroid hormone receptor with retinoid X receptor. J. Biol. Chem. 271, 28199-28205 PMCID: 8910436
3. Adams, M., **Reginato, M. J.,** Shao, D., Lazar, M. A., and Chatterjee, K. (1997) Transcriptional activation by peroxisome proliferator-activated receptor g is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. J. Biol. Chem. 272, 5128-5132. PMCID: 9030579
4. **Reginato, M. J.,** Krakow, S. L., Bailey, S. T., and Lazar, M. A. (1998) Prostaglandins promote and block adipogenesis through opposing effects on PPARg. J. Biol. Chem. 273, 1855-1858. PMCID: 9442016
5. Shao, D., Rangwala, S. M., Bailey, S. T., Krakow, S. L., **Reginato, M. J.,** and Lazar, M. A. (1998) Interdomain communication regulating ligand binding by PPARg Nature 396, 377-380. PMCID: 9845075
6. **Reginato, M. J.,** Bailey, S. T., Krakow, S. L., Minami, C., Ishii, S., Tanaka, H., and Lazar, M. A. (1998) A potent antidiabetic thiazolidinedione with unique PPARg-activating properties. J. Biol. Chem. 273, 32679-32684. PMCID: 9830009
7. Debnath, J., Mills, K. R., Collins, N., **Reginato, M. J.,** Muthuswamy S., and Brugge, J. S. (2002) The role of apoptosis in creating and maintaining luminal space within normal and oncogene expressing mammary acini. Cell 111, 29-40. PMCID: 12372298
8. **Reginato, M. J.,** Mills, K. R., Paulus, J. K., Lynch, D. K., Sgroi, D.C., Debnath J., Muthuswamy S., and Brugge, J. S. (2003) Integrins and EGFR coordinately regulate the pro-apoptotic protein Bim to prevent anoikis. Nature Cell Biol. 5, 733-740. PMCID: 12844146
9. Mills. K. R., **Reginato, M. J.,** Debnath, J., Queenan, B., and Brugge, J. S. (2004) Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is required for induction of autophagy during lumen formation in vitro. Proc. Natl. Acad. Sci. USA, 101, 3438-3443. PMCID: 14993595
10. **Reginato, M. J.,** Mills, K. R., Becker, E. B., Lynch, D. K., Bonni, A., Muthuswamy S., and Brugge, J. S. (2005) Bim regulation of lumen formation in cultured mammary epithelial acini is targeted by oncogenes. Mol. Cell. Biol. 25, 4591-4601. PMCID: 15899862
11. Collins, N., **Reginato, M. J.,** Paulus, J. K., LeBear, J., and Brugge, J. S. (2005) G1/S cell cycle arrest provides anoikis resistance through Erk-mediated Bim suppression. Mol. Cell. Biol. 25, 5282-5291. PMCID: 15923641
12. **Reginato, M. J.** and Muthuswamy, S. K. Illuminating the Center: Mechanisms Regulating Lumen Formation and Maintenance in Mammary Morphogenesis. (2006) J. Mammary Gland Bio & Neoplasia, 11:205-11. PMCID: 17115263

Research Support.

Ongoing Research Support:

Project Title:	**Role of nutrient sensor O-GlcNAc transferase in regulating cancer**
Sponsor:	NIH/NCI, 1R01CA155413-01
Project Period:	2/11-1/16
P.I:	Mauricio Reginato

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Fernando U. Garcia, M.D.	Professor in Pathology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Universidad Peruana Cayetano Heredia	MD	1981	Medicine
University of Kansas, Kansas City, Kansas		1982-1986	Resident in Pathology
Vanderbilt University, Nashville, Tennessee		1986-1987	Fellow in Surg Pathology
Vanderbilt University, Nashville, Tennessee		1987-1988	Chief Resident
Vanderbilt University, Nashville, Tennessee		1988-2000	Instructor in Pathology

Professional Experience:

1987-1990 Instructor, Department of Pathology, Vanderbilt University, Nashville, Tennessee.
1988-1990 Consultant, Laboratory Services, Veterans Administration Medical Center, Nashville, Tennessee.
1990-1991 Clinical Instructor, Pathology Department Kansas University, Kansas City, Kansas.
1990-1994 Director, Tissue Culture Core Facility, Pathology Department, Kansas University Medical Center, Kansas City, Kansas.
1991-1994 Medical Director, Cytopathology Laboratory Pathology Department, Kansas University, Kansas City, Kansas.
1991-1994 Assistant Professor, Pathology Department Kansas University, Kansas City, Kansas.
1992-1994 Consultant, Laboratory Services, Veterans Administration Medical Center, Leavenworth, Kansas.
1992-1994 Director of Dermatopathology, Kansas University, Kansas City, Kansas.
1994-1994 Director of Image Analysis, Kansas University, Kansas City, Kansas.
1995-2000 Associate Professor MCP-Hahnemann University Philadelphia, Pennsylvania.
1997-2001 Medical Director, Immunopathology Laboratory, MCP-Hahnemann University, Hahnemann University Hospital.

2001- 2005 Medical Director, Immunopathology, Department of Pathology, Drexel University College of Medicine Hahnemann Hospital, Philadelphia.
1997- Medical Director, Image Analysis Laboratory, Department of Pathology, Drexel University College of Medicine Hahnemann Hospital, Philadelphia.
1997- Medical Director, Tissue Procurement Core Laboratory, Department of Pathology, Drexel College of Medicine, Philadelphia.
1998- Regional Director of Anatomic Pathology, Department of Pathology, Drexel University College of Medicine, Philadelphia.
2000-Present Professor of Pathology, Drexel University College of Medicine Philadelphia, Pennsylvania.
2001-2005 Medical Director & Service Chief, Graduate Hospital
2005- Medical Director & Service Chief, Hahnemann Hospital

Certifications:

1981	Peru Medical License (13087)
1981	Educational Commission for Foreign Medical Graduates Certificate (329-894-0)
1984	Visa Qualyfing Examination (VQE)
1985	Federal Licensure Examination (FLEX)
1987	Tennessee Medical License (MD 17629) Not Active
1987	Diplomate of the American Board of Pathology in Anatomic Pathology
1990	Kansas Medical License (4-23066) Not Active
1993	Diplomate of the American Board of Pathology in Cytopathology
1995	Pennsylvania Medical License (MD-054895-L)

Publications (last six years)

1. Ohkia, A, Hu, Y, Wang, M, **Garcia, FU**, Stearns, ME. Evidence for prostate cancer-associated diagnosis marker-1: Immunohistochemistry and *in-situ* hybridization studies. Clin Cancer Res 2004;10: 433-440.
2. Narins Sc, Park, EH, Ramakrishnan, R, **Garcia, FU**, Diven, JN, Balin, BJ, Hammond, CJ. Functional characterization of Na+/H+ exchanges in primary cultures of prairie dog gallbladder. J Membrane Biol 2004;197:123-134.
3. Wang, JY, Ho, T, Trojanek, J, Chintapalli, J, Grabacka, M, Stoklosa, T, **Garcia, FU**, Skorski, T, Reiss, K. Impaired homologous recombination DNA repair and enhanced sensitivity to DNA damage in prostate cancer cells exposed to anchorage-independence. Oncogene 2005 00, 1-11
4. Leon, ME, Hou, JS, Galindo, LM and **Garcia, FU**. Fine needle aspiration of adult small round-cell tumors studied with flow cytometry. Diagnostic Cytopathology 2004;31:147-154.
5. Wang,JY ,Ho, T., Trojanek,J,, Chintapalli,J, Grabacka,M, Stoklosa,T, **Garcia, FU.** Skorski,T and Reiss,K. Impaired homologous recombination DNA repair and enhanced sensitivity to DNA damage in prostate cancer cells exposed to anchorage-independence. Oncogene (2005) 24, 3748-3758.
6. Popnikolov, NK, Cavone, SM, Schultz, PM, **Garcia, FU**. Diagnostic utility of p75 neurotropin receptor (p75 ^{NTR}) as a marker of breast myoepithelial cells. Modern pathology, 2005, 18:1535-1541.
7. Chen, X, Zhuo, Salim,S and **Garcia, FU**. Intraprostatic spermatozoa: Zonal distribution and association with atrophy. Human Pathology, 2006 37(3):345-351.
8. Petushi, S, **Garcia, FU**, Haber, MM, Katsinis, C , Tozeren, A. Large-scale computations on histology images reveal grade-differentiating parameters for breast cancer. *BMC Medical Imaging* 2006, **6:**14 doi:10.1186/1471-2342-6-14. http://www.biomedcentral.com/1471-2342/6/14
9. 15. Raab, SS, Geisinger, KM, Grzybicki, DM, Janosky, JE, **Garcia, FU**, Wojcik, EM, Dahmoush, L, Stone, CH, Meier, FA, Zarbo, RJ. Use of a New Method in Reaching Consensus on the Cause of Cytologic-Histologic Correlation Discrepancy. Am J Clin Pathol 2006;126(6):836-842.
10. Mahmoodi, M, Zhang, S, Salim, S, Hou, JS, **Garcia, FU**. Lipofucsin pigment can be used as a prognostic marker in prostatic adenocarcinoma. Annals in Diagnostic Pathology, 2006; 10(5):257-262.
11. Hu,Y ,Wang, M, Veverka, K, **Garcia, FU** and Stearns, ME. The ABCA5 Protein: A Urine Diagnostic Marker for Prostatic Intraepithelial Neoplasia. Clinical Cancer Research 13, 929-938, February 1, 2007.
12. **Garcia, FU,** Urbanska, K, Koltowski, L, Reiss, K Sell C. IGF-I produced by seminal vesicles: relationship to intraepithelial basal cell hyperplasia in the prostate. *Clinical Cancer Research* 13, 3140-3146, June 1, 2007
13. Vamvakidou, AP, Mondrinos, MJ, Petushi, SP, **Garcia, FU**, Lelkes, PI, Tozeren, A. Heterogenous breast tumoroids: An in vitro assay for investigating cellular heterogeneity and

drug delivery. J Biomol Screen 2007 Feb;12(1):13-20.
14. Maraldo,D, **Garcia, FU**, Mutharasan, R. Method for quantification of a prostate cancer biomarker in urine without sample preparation. Anal Chem 2007 Oct 15;79(20):7683-90.
15. **Garcia, FU**, Haber, MM, Chen X. Prostatic basal cells in the peripheral zone and transitional zones: Zonal variation in morphology and in immunophenotype. Prostate 2007 Nov 1;67(15):1686-92.
16. **Garcia, FU**, Sharma, M, Nagle, D, Haber, MM. Increase sensitivity of anal cytology in evaluation of internal compared with external lesions. Acta Cytologica 2007, 51:893-899.