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14. ABSTRACT

The project deals with the identifying treatment and response targets for *Helicobacter*-associated gastric cancer. The purpose is to discover novel druggable targets by testing efficacy of approved compounds. An additional purpose is to identify predictors of treatment response. To accomplish the goals of our project we are using gastric cancer patient samples, patient-derived xenograft (PDX), and patient-derived orthotopic xenograft (PDOX) models. In addition, the work makes use of gastric cancer patient samples stored at our UCSD's Moores Cancer Center Biorepository core. We have successfully established a gastric cancer PDX model and show that the tumor gastric tissue in the PDX model express the same genes as the original tumor. We report on expression of cancer markers such as the carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) and the tumor-associated glycoprotein 72 (TAG-72) in patient gastric tumors. These cancer markers were undetectable in normal gastric tissue.

15. SUBJECT TERMS

Helicobacter pylori, gastric cancer, patient-derived xenograft (PDX), patient-derived orthotopic xenograft (PDOX), differentially expressed genes (DEGs).

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1. INTRODUCTION:

The project deals with the identification of treatment and response targets for *Helicobacter*-associated gastric cancer. This work leverages our findings from our model of gastric cancer[1,2] to discover novel druggable targets by testing efficacy of approved compounds and to identify predictors of treatment response using gastric cancer patient samples, patient-derived xenograft (PDX), and patient-derived orthotopic xenograft (PDOX) models. In addition, the work makes use of gastric cancer patient samples stored at the tissue biobank at the UCSD's Moores Cancer Center Biorepository core.

2. KEYWORDS:

Helicobacter pylori, gastric cancer, patient-derived xenograft (PDX), patient-derived orthotopic xenograft (PDOX), differentially expressed genes (DEGs).

3. ACCOMPLISHMENTS:

3.1: What are the major goals of the project?
The major goals of the project are to deliver drugs to slow or stop the progression of gastric cancer and to determine patients likely to respond well to a drug.

The specific aims of the project are:

Specific Aim 1: Test efficacy of compounds with approved indications for human use or currently in clinical trials, that potentially target pathways associated with select differentially expressed genes (DEGs) (casp1, Ido1, psmb8, psmb9, psmb10, and mmp13) in patient-derived xenograft (PDX) and patient derived orthotopic xenograft (PDOX) models of gastric cancer.

Specific Aim 2: Determine immune related DEGs including Tnfα, Cxcl9, Cxcl5, Cxcl10, B2m, Cd74, and Cd247 as predictors of response to gastric cancer treatment in patient samples.

3.2: What has been accomplished under these goals.

Gene expression using gastric tissue samples stored at our biorepository (UCSD's Moores Cancer Center Biorepository core). Freshly-frozen patient gastric samples (8 adenocarcinomas and 2 normal) from our MCC biorepository core were ground using a tissue multi-pulverizer and then homogenized by a QIAshredder (Qiagen) followed by RNA isolation and qRT-PCR as described in our previous studies.[1,3] We found that interferon-stimulated genes (ISGs) including *GBP2, IRF1, OAS2, B2M*, and *MX1*) were upregulated in patient gastric cancer tissues (**Fig. 1**), similar to what we observed in mice that developed high-grade gastric dysplasia.[2] As an example, *GBP2* has been shown to be highly expressed in human esophageal squamous cell carcinoma.[4] High expression of *GBP2* has been associated with higher proliferation.[5] Notably, we observed enhanced gastric epithelial cell proliferation in our mouse model of gastric cancer, which was associated with acquisition of a stem-cell phenotype as evidenced by expression of stem-cell markers.[6] We propose that expression of ISGs we observed in

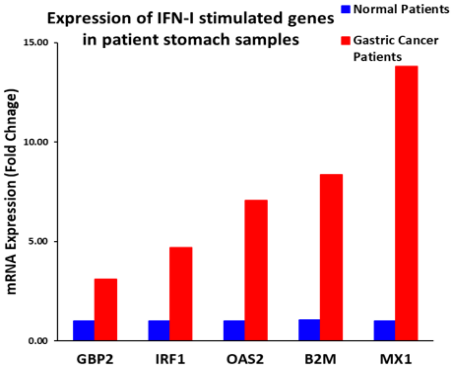

Figure 1: IFN-I stimulated genes were upregulated in gastric cancer patient stomach samples obtained from our biorepository core. Freshly frozen patient stomach samples were pulverized and homogenized followed by qRT-PCR.

both the mouse model and patient gastric tissues represent molecular changes associated with the progression of gastric cancer.

Analysis of patient gastric tissue freshly obtained immediately after surgery. We have collected fresh gastric tissue from 11 patients. All 11 gastric tumor tissue samples were implanted into nude mice and of these two successfully grew and have been reimplanted (propagated) into another set of nude mice (2nd generation). Five gastric tumor tissue samples from patients implanted in nude mice are still growing. Two gastric tissue samples implanted in mice stopped growing. Figure 2 is an example of a successfully established gastric cancer PDX model. We have recently started analyzing PDX tumors that have been reimplanted into nude mice as 2nd generation (KG1 and KG2). Western blot indicated differential expression of some of the well-known cancer markers including the carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) and the tumor-associated glycoprotein 72 (TAG-72) between the two PDX tumors. Expression of TAG-72 and CEACAM family members have been associated with gastric cancer.[7-10] There was no expression of CEACAM 1, CEACAM 3, CEACAM 5, and TAG-72 in normal human (N. H) or mouse (N. M) gastric tissue (Fig. 3).

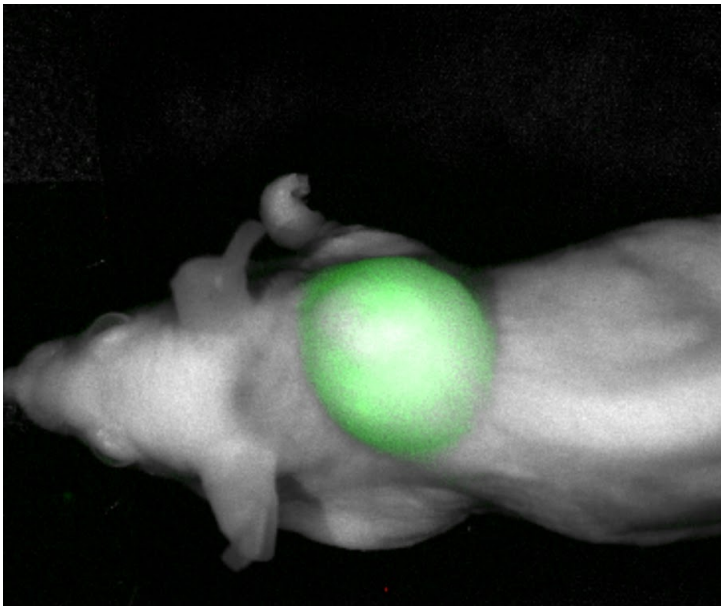

Figure 2: PDX of KG1. A patient gastric tumor (labelled KG1) in a nude mouse was injected with 50 µg of SAB-800 (antibody to CEACAM 1/3/5 conjugated with 800 NIR dye).

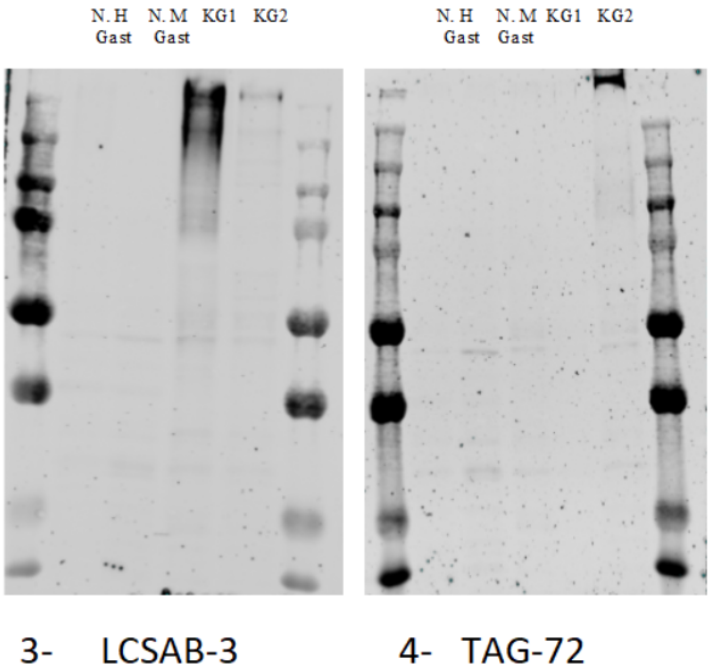

Figure 3: Expression of CEACAMs and TAG-72 in patient tumor tissue. Protein was extracted from tumors of PDX nude mice followed by Western blot using antibodies to identify CEACAMs and TAG-72. Normal human and mouse gastric tissue was used as controls.

We have yet to start testing efficacy of our select compounds that potentially target pathways linked with selected DEGs (casp1, Ido1, psmb8/9/10, and mmp13) that were associated with gastric cancer progression in our gastric cancer model.[1] This part of the project has been delayed due to reduced patient surgeries due to the COVID-19 pandemic. Patients have now started scheduling appointments and the University is now at 50% work capacity. We will therefore be able to make up for lost time.

3.3: What opportunities for training and professional development has the project provided.
Nothing to Report.

3.4: How were the results disseminated to communities of interest?
Nothing to Report.

3.5: What do you plan to do during the next reporting period to accomplish the goals.

With more patients coming in for gastric cancer surgeries, we plan to complete generation of PDX gastric cancer models. This will be followed by drug treatments targeting select pathways. At the same time, we will continue evaluating select immune related genes in PDX models and in patient tumor gastric tissues stored at our biorepository.

4. IMPACT:

4.1: What was the impact on the development of the principal disciple(s) of the project?
Nothing to Report.

4.2: What was the impact on other disciplines.
Nothing to Report.

4.3: What was the impact on technology transfer?
Nothing to Report.

4.4: What was the impact on society beyond science and technology?
Nothing to Report.

5. CHANGES/PROBLEMS:

5.1: Changes in approach and reasons for change.
There are no changes in the approach and therefore, nothing to report.

5.2: Actual or anticipated problems or delays and actions to resolve them.
Due to the COVID-19 pandemic we were not able to harvest many gastric tumor biopsies as we had anticipated. We adjusted by focusing mostly on using freshly frozen gastric tumor tissues stored at our biorepository core. Gastric cancer patients have started coming to our clinic for surgeries and therefore we will eventually get to the target of 25 patients.

5.3: Changes that had a significant impact on expenditures.
Nothing to Report.

5.4: Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.
Nothing to Report.

5.5: Significant changes in use or care of human subjects.
Nothing to Report.

5.6: Significant changes in use or care of vertebrate animals.
Nothing to Report.

5.7: Significant changes in use of biohazards, and/or select agents.
Nothing to Report.

6. PRODUCTS:

6.1: Publications, conference papers, and presentations
Nothing to Report

6.2: Website(s) or other Internet site(s)
Nothing to Report

6.3 Technologies or techniques
Nothing to Report

6.4: Inventions, patent applications, and/or licenses
Nothing to Report

6.5: Other products
Nothing to Report

7. PARTICIPANTS & OTHER COLLBORATING ORGANIZATIONS:

7.1: What individuals have worked on the project?

Name:	Marygorret Obonyo
Project Role:	PI
Nearest person month worked:	3.6
Contribution to Project	Dr. Obonyo was involved in the design of experiments and overall supervision of the project.
Funding Support	
Name:	Michael Bouvet
Project Role:	Investigator
Nearest person month worked:	0.6
Contribution to Project	Dr. Bouvet alongside his lab staff assistant, Siamak Amirfakhri have generated the gastric cancer patient-derived xenograft (PDX) model.
Funding Support	
Name:	Kaitlyn Kelly
Project Role:	Investigator
Nearest person month worked:	0.6
Contribution to Project	Dr. Kelly has provided both involved and uninvolved patient gastric cancer specimens.
Funding Support	
Name:	Siamak Amirfakhri
Project Role:	Staff Research Assistant
Nearest person month worked:	2.4
Contribution to Project	Under supervision of Dr. Bouvet, Siamak Amirfakhri has generated the gastric cancer PDX model.
Funding Support	
Name:	Michael Turner
Project Role:	MD Fellow
Nearest person month worked:	1.2

Contribution to Project	Dr. Turner has performed work related to the generation and maintenance of the gastric cancer PDX model.
Funding Support	T32 NIH Training grant
Name:	Jonathan Hernandez
Project Role:	Staff Research Assistant
Nearest person month worked:	6
Contribution to Project	Under the supervision of Dr. Obonyo, Jonathan Hernandez has performed RNA extraction and RT-qPCR of patient gastric tissue fresh after surgery or stored at our biorepository.
Funding Support	

7.2: 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.

7.3: What other organization were involved as partners?
Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS
Nothing to Report.

9. APPENDICES:
Nothing to Report.

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