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**14. ABSTRACT**

Cancer of the large intestine (colon and rectum) ranks the third most common and second most deadly in the United States. It affects Service members and their families as much as the civilian population. Sadly, approximately half of these patients develop metastatic disease (*i.e*. spread), most frequently to the liver, resulting in premature deaths. Scientific advances have led to a much better appreciation of the genetic changes (*e.g.* mutations) associated with the development and progression of colorectal cancer (CRC). Like most cancers, CRC differ from one another in their genetic make-up, clinical behavior, and treatment response. This holds true even within one tumor, which makes it challenging for physicians to choose the best treatment for individual patients. Despite the promise of personalized medicine to define the precise genetic landscape of each cancer, there remains an enormous gap between our knowledge of the genomic alterations harbored by a tumor, and how these changes affect the biology of the cancer and its response to drugs. In other words, there is a missing link between the scientific world of cancer genetics and the clinical world of therapeutic decisions. The outdated "one- size-fits-all" paradigm of prescribing chemotherapy according to conventional diagnosis must be abandoned. To bring us closer towards the goal of tailoring 'effective' therapy for individual cancer (*i.e.* 'personalized' oncology), we will establish a new platform for testing drug sensitivity outside the body, using tissues directly obtained from the patient's cancer in the form of a slice culture. Pieces of human cancers will be kept alive in an incubator for days without significant detriment to their viability. During this period, we will test a variety of drugs and cell-based therapies to determine their effects on the specific tumors. Unlike other forms of human cancer models, our Tumor Slice Culture (TSC) system utilizes a standardized easy-to-follow protocol to come up with results that are reproducible and clinically relevant in a very short timeframe compared to other methods (*e.g*. 2 weeks vs. 3-6 months). Consequently, we feel strongly that our TSC platform will aid physicians in their decision making and lead to more effective cancer therapy, while minimizing the toxicities and expense of ineffective drugs. The goals of this proposal are to optimize and validate the assay for traditional chemotherapy, and to explore the utility of our platform for testing 'targeted' therapies and immunotherapies, all of which are showing great promise in the treatment of human cancers.

While our approach applies to all forms of cancers listed in the FY15 PRCRP topic areas, this proposal focuses on CRC as proof-of-principle to demonstrate the versatility of TSC to assess various forms of systemic therapies. We have assembled a team of surgeons, medical oncologist, bioengineer, immunologist, system biologist and basic pharmacologist across three institutions to bring this technology to the bedside. The key innovations of our work are 1) the development of a quantitative metric to evaluate TSC and to compare results across different samples and patients, 2) the ability to analyze all forms of systemic treatment for any one tumor and to correlate the findings with clinical responses and molecular data, and 3) the adoption of a microfluidic device for high efficiency testing. Upon completion of this proposal, our TSC platform will be ready for clinical application.

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## INTRODUCTION

Our investigation aims to develop, optimize, and apply the use of tissue slice culture as an avatar for testing sensitivity of human tumors to various forms of cancer therapies. This application focuses on colorectal cancer to illustrate how conventional chemotherapy affects colorectal liver metastases and to explore novel immunotherapeutic approaches in microsatellite-stable disease.


## KEYWORDS

Organotypic, colorectal cancer, drug sensitivity, chemotherapy, immunotherapy

**ACCOMPLISHMENTS**

A. What were the major goals of the project?

The goal of our research is to advance our ability to precisely tailor therapy for solid tumors based on an ex-vivo tumor slice culture (TSC) platform as a novel avatar of personalized oncology. The age of genomic medicine has brought tremendous hope to cancer patients, but the way in which this information gets translated to a clinical decision represents a major knowledge gap facing the oncologist. We now know that tumors with the same genetic mutation can have highly variable responses to targeted therapy. The lack of a functional assay with a quick turnaround time is the Achilles heel of personalized oncology. The objective of this proposal is to establish a robust, efficient, reproducible platform to interrogate drug sensitivity and to correlate the results with clinical and molecular data.

The 3 Projects will address the utility of TSC in testing cytotoxic chemotherapy, targeted kinase inhibitors and immunotherapy in CRC liver metastases (CRLM).

B. What was accomplished under these goals?

*1. Tumor Slice Culture as Predictor of Chemosensitivity Investigators: Yeung, Lin, Folch*

One of the main goals was to establish a standardized protocol to assess drug sensitivity. Towards this end, we studied a large (>100) number of human solid tumors to optimize the steps of procurement, tissue handling, slicing, culturing and viability readouts. We summarized our findings in a manuscript that has been accepted for publication (*Kenerson et al. Tumor Slice Culture as a Biologic Surrogate of Human Cancer. Ann Trans Oncol. In press* - see Appendix).

Next, we used our slice culture platform to investigate the response of CRLM to conventional cytotoxic chemotherapy. Not surprisingly, we found that different tumors responded differently to the two drug combinations that represent first-line treatment for CRLM: FOLFOX and FOLFIRI. Based on bulk RNAseq of treated slices, changes in proliferative pathways correlated with clinical response to these regimens, suggestive of the potential utility of in vitro drug testing using human slice culture to guide therapeutic choice. More importantly, we analyzed the response of individual cellular compartment to chemotherapy using single-cell RNAseq. In our experience of 8 cases, we identified two distinct clusters of cancer cells that differ in their response to FOLFIRI. We found that cancers with stem-like features were more sensitive to FOLFIRI. Remarkably, these drugs induced an interferon response along with suppression of PD-1 ligands leading to T-cell activation. Conversely, FOLFIRI stimulated the expression of TIM- 3 ligands in non-stem-like CRLMs, which increased their sensitivity to $\alpha$-TIM-3 checkpoint blockade. Our findings led us to explore the relationship between tumor expression of immune checkpoints and their clinico-pathologic-molecular phenotype, and the influence of chemotherapies on the susceptibility to immune checkpoint inhibition. In a case of a PD-L1$^{-}$;Gal-9$^{+}$ CRLM, the combination of TIM-3 blockade with FOLFIRI led to a synergistic effect. Given the current limitation of the role of immunotherapy in MMR-proficient colorectal cancer, our findings highlight the rational use of combination chemo-immunotherapy to improve durable response and clinical outcome. Details of our findings are summarized in a manuscript (*Jabbari et al. Chemotherapy modulates tumor immune microenvironment of human metastatic colorectal carcinoma*. In preparation). Note: The work related to single-cell RNA sequencing was contributed by Qiang and his team at the Institute for Systems Biology.

Finally, the team headed by Folch in Bioengineering was able to fabricate microfluidic devices by 3D-printing and we were successfully in treating CRLM slices with drugs using this device with fluorescent LIVE-DEAD assay as readout (*Rodriguez et al. A Microfluidic Platform for Functional Testing of Cancer Drugs on Intact Tumor Slices. Lab on Chip* In press). This allows for higher throughput, which greatly enhances our ability to derive drug response curves for single and combination therapies.

*2. Kinase inhibition in metastatic colorectal carcinoma Investigators: Qiang, Ong, Yeung*

The main objectives of this Project were to 1) To identify 'druggable' kinases activated in individual CRCs using a combination of kinomic, genomic, and transcriptomic analyses; 2) To test the effects of kinase inhibitors on CRC TSCs, and 3) To monitor cytotoxicity of inhibitors in normal liver slice cultures.

For over a year, we encountered problems with our phosphoproteomics workflow that significantly affected our ability to identify phosphopeptides from complex mixtures. For example, before the issue arose, we typically identified 5000-6000 phosphopeptides from 500 microgram starting material but were down to 2000-3000 per run. Seeking to address these issues before analyzing precious clinical samples, we tested multiple parameters to try to address the issues of decreased sensitivity. This included a full replacement of all reagents and materials, including phosphoenrichment reagents, solvents, buffer. We have tested alternate

protein digestion protocols, different sources of protein from cell lines and even phosphosamples from other labs. We have employed different operators and mass spectrometer instrument platforms. Despite considerable effort and investment of time, we have still been unable to completely resolve these issues. We have decided to delay analyses of the clinical samples till the issues with our analytical platform have been addressed. Instead, we dedicated more effort in the transcriptomic analyses of human CRLM, especially as it relates to the response following exposure to chemotherapy as outlined in Aim 1 above.

*3. Immunotherapy in CRLM Investigators: Pillarisetty, Katz, Crispe*

The immune microenvironment is particularly challenging to interrogate in human solid cancers. Using pancreatic cancer as a model, we showed that the various immune cells inherent in the tumor remain viable and functional in fresh tumor slices in culture (Jiang et al. 2017) [1]. Be extending this platform to CRLM, we were able to track the effects of various strategies to invigorate tumor infiltrating lymphocytes. Specifically, the combination of PD-1 and CXCR4 blockade led to synergistic cell kill in concert with T-cell activation as we have reported in pancreatic cancer (Seo et al. 2019) [2]. Another strategy that we explored was to target the immunosuppressive effects of IL-10. Based on the TCGA database, we found that higher IL-10 transcript levels in colorectal cancer correlated with reduced overall survival and disease-specific survival. To determine the effects of IL-10 blockade, we exposed CRLM tumor slices to an anti-IL-10 or control antibody and found evidence of enhanced cell death when IL-10 is blocked. To further dissect the underlying mechanism, we used single-cell RNAseq to deconstruct the cellular response focusing on the immune compartments. The results show that IL-10 was exclusively expressed in tumor associated macrophages and not T cells, while its receptor was present in both macrophages and T cells. Overall, changes in gene expression following IL-10 blockade highlight an increase in T cell activity along with a decrease in immunosuppression by Tregs and macrophages. Moreover, we found evidence of TIM-3 down-regulation, which together led to anti-tumor immune response (manuscript in preparation).

Last, we tested the effects of CEA CAR-T cell therapy with and without IL-10 inhibition in CRLM. CFSE-labelled CAR-T cells were found to infiltrate tumor slices within 24 hours and remained viable. CEA CAR-T cells alone elicited a response with a significantly high rate of apoptosis compared with control CAR T cells. Moreover, the addition of anti-IL-10 antibody led to increased number of CEA CAR-T cells in close proximity of tumor cells, which exhibited evidence of apoptosis. Together, our findings suggest that IL-10 play a crucial role in tumor evasion of CRLM, which can be therapeutically targeted to improve anti-tumor response by either endogenous T cells or exogenous CEA CAR-T cells.

C. What opportunities for training and professional development has the project provided?

The development of our tumor slice culture platform has attracted a lot of attention locally, and there has been a constant flux of trainees (students, residents, and fellows) who have spent time in our labs to work on various aspects of the projects.

D. How were the results disseminated to communities of interest?

Results from our investigations have and will be disseminated to the scientific community through publications in peer-reviewed journals and by scientific presentations at local and national meetings.

E. What do you plan to do during the next reporting period to accomplish the goals?

This is the final report.

**IMPACT**

A. What was the impact on the development of the principal discipline(s) of the project?

The central theme of our research is to make use of organotypic slice culture to investigate the behavior of human colorectal cancer in response to treatments. Our studies have collectively refined and optimized the slice culture platform for in vitro testing of sensitivity to chemotherapy and immunotherapy. The latter is particularly impactful given the scarcity of tumor models that recapitulate the human immune microenvironment. We believe that our technique holds promise for screening compounds to determine drug response in any human solid tumors.

B. What was the impact on other disciplines?

Nothing to report

C. What was the impact on technology transfer?
While too early to tell, the potential of using an organotypic culture of human cancer to predict drug sensitivity may have widespread applications. Future studies will address the clinical translation of our platform.

D. What was the impact on society beyond science and technology?
If one can more accurately predict treatment response, doctors will be able to prescribe drugs that are more effective resulting in better outcome, reduced cost and toxicities, and improve quality of life.

**CHANGES/PROBLEMS**

A. Changes in approach and reasons for change
No significant change in our approach with the exception of a more detailed analysis of treatment response following chemotherapy.

B. Actual or anticipated problems or delays and actions or plans to resolve them
As noted above, Project 2 presented significant technical challenges stemming from our inability to obtain sufficient reads in the proteomic workflow when complex primary human tissues were used. We are still in the process of deconstructing the problem to find solutions. Instead of focusing solely on proteomics, we analyzed the transcriptome instead.

C. Changes that had a significant impact on expenditures
Overall budget did not change, but some of the funds allotted for proteomics were shifted to RNAseq analyses.

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

**PRODUCTS**

A. Publications, conference papers, and presentations
a. Kenerson et al. Tumor Slice Culture as a Biologic Surrogate of Human Cancer. Ann Trans Oncol. In press
b. Rodriguez et al. A Microfluidic Platform for Functional Testing of Cancer Drugs on Intact Tumor Slices. Lab on Chip In press

B. Websites or other internet sites
Nothing to report

C. Technologies or techniques
Nothing to report

D. Inventions, patent applications and/or licenses
Nothing to report

E. Other Products
Nothing to report

**PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

A. What individuals have worked on the project?
Raymond Yeung: PD/PI No change
Albert Folch: Co-I No change
Shao-En Ong: Co-I No change
Qiang Tian: Co-PI Dr. Tian transitioned his role to Dr. Wei during the last 6 months of the Award as Dr. Tian took up a different position in China.
Venu Pillarisetty: Co-PI No change
Ian Crispe: Co-I No change

| | |
|---|---|
| Steven Katz: Co-I | No change |
| Ed Lin: Co-I | Dr. Lin departed the Institution after Year 1 |

B. 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

C. What other organizations were involved as partners?

Institute for Systems Biology, Seattle (Qiang, Wei), and Roger Williams Medical Center (Katz) have been partnering Institutions from the start without change. Both of these partnerships were included in our original proposal without change.

**SPECIAL REPORTING REQUIREMENTS**

Collaborative awards

This Translational Team Science Award involves two collaborative PI (Qiang/Wei and Pillarisetty) and one overall PI (Yeung) that brought three institutions together (University of Washington, Institute for Systems Biology, and Roger Williams Medical Center). As a Team, each PI is submitting a duplicative report representing all of the work.

**APPENDICES**

Manuscript in press:

Kenerson et al. Tumor Slice Culture as a Biologic Surrogate of Human Cancer. Ann Trans Oncol. In press

Rodriguez et al. A Microfluidic Platform for Functional Testing of Cancer Drugs on Intact Tumor Slices. Lab on Chip In press

References:

1. Jiang X, Seo YD, Chang JH, et al. Long-lived pancreatic ductal adenocarcinoma slice cultures enable precise study of the immune microenvironment. Oncoimmunology 2017;6:e1333210.
2. Seo YD, Jiang X, Sullivan KM, et al. Mobilization of CD8+ T cells via CXCR4 blockade facilitates PD-1 checkpoint therapy in human pancreatic cancer. Clin Cancer Res 2019.