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TITLE: **Tumor Slice Culture: A New Avatar in Personalized Oncology**

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14. ABSTRACT The goal of this research is to advance our ability to precisely tailor therapy for solid tumors based on an <i>ex-vivo</i> 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. <i>The lack of a functional assay with a quick turnaround time is the Achilles heel of personalized oncology.</i> 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.					
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Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	7
5. Changes/Problems.....	8
6. Products.....	8
7. Participants & Other Collaborating Organizations.....	8
8. Special Reporting Requirements.....	9
9. Appendices.....	NA

Annual Report 2018: Tumor Slice Culture: A new avatar for personalized oncology

1. **INTRODUCTION:** The goal of this 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 objective of this proposal is to establish a robust, efficient, reproducible platform to interrogate the response of a given tumor to drugs (cytotoxics, kinase inhibitors, immune modulators) and adoptive cellular immunotherapy using human samples.
2. **KEYWORDS:** organotypic, colorectal, metastases, in vitro, drug sensitivity, slice culture, tumor infiltrating lymphocytes (TIL), CAR-T (chimeric antigen receptor T cells), PD-1, kinobeads, RNAseq, kinases.
3. **ACCOMPLISHMENTS:**

Project 1: Tumor Slice Cultures as Predictor of Chemosensitivity

The work in Project 1 was conducted at the University of Washington and Institute for Systems Biology involving the P.I., Raymond Yeung, and co-PI, Qiang Tian.

Specific Aim 1: Testing chemosensitivity using TSC

Major Task 1: Patient selection and enrollment

Over the 12 months, we enrolled an additional 16 patients from the UWMC Liver Tumor Clinic with a diagnosis of colorectal carcinoma with liver metastases who underwent surgical resection. At this time, we have sufficient samples to optimize our platform.

Major Task 2: Tumor slice cultures

Overall, we have completed >100 cases of tumor slice culture, many of which were derived from metastatic colorectal cancer. A standardized protocol has been developed so that samples from different tumors or tissues can be compared in term of their growth in vitro. From our experience, we found that baseline viability and growth of the tumor slices varied greatly, depending on histology and prior therapies. Specifically, we performed multi-variate analyses to correlate MTS values with clinical and pathologic parameters. As expected, baseline MTS correlates with pathologic assessment of tumor viability, thus validating our assay. We extended the use of this assay to examine tumor heterogeneity and found that we could detect significant changes in MTS values of the tumor within a few millimeters; whether these observed differences translate to their biologic behavior or response to treatment is not yet known.

To improve through-put of our platform, we began exploring the potential of using a microfluidic device made by the Folch's lab. They have optimized their perfusion chamber using a mouse glioma model to accommodate up to 22 channels of perfusion. In preliminary studies, we were able to make use of the device to discriminate live and dead cells (Fig. 1).

Major Task 3: Drug treatments and responses

Following standardization of our treatment protocol, we examined a series of colorectal cancer for their relative sensitivity to two standard-of-care regimens consisting of conventional chemotherapies: FOLFOX and FOLFIRI. Up to now, the selection of first-line treatment is largely empiric, each having ~40% chance of response. In our in vitro assay, we observed 3 patterns of response: FOLFOX being better, equal, or worse compared to FOLFIRI; this is broadly consistent with the clinical observation that

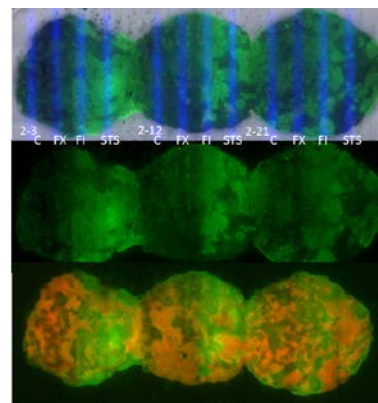


Figure 1. Example of drug sensitivity testing using a microfluidic device on three colorectal cancer slices. Green: liver; orange: dead.

colorectal cancers show differential sensitivity to the two regimens. We came across one unique example of two metastases from one patient showing opposite responses to chemotherapies; one showing regression while the other progression. When tested in vitro, we were able to recapitulate the divergent in vivo response. These and other examples highlight the potential for using the tumor slice culture to guide therapeutic choices.

Specific Aim 2: Correlation with clinical and molecular data

Major Task 1 and 2: Molecular data analyses

We have made striking progress in the past year employing tumor slice cultures (TSCs) of CRCLM as a proxy for evaluating responses of drug treatments, in particular two chemotherapeutic regimens FOLFOX and FOLFIRI. In particular, TSCs were treated with each regimen for three days in vitro, and were subjected to transcriptomic analysis (at both bulk and single cell levels) before and after treatments to establish molecular networks impacted by the drug treatments. We have generated bulk time series RNA-seq data of five tumor tissues from four CRC patients with a total of 65 transcriptomes. In addition, single cell RNA-seq data from 7,580 individual cells have been generated from eight tumor samples. Computational analysis of these single cell RNA-seq data employing K-mean clustering algorithm digitally identified 10 distinct tumor cell subpopulations including cells of both colon and liver origins, tumor microenvironment, cancer stem cells and various immune cells. Key molecular networks defining each of the cell populations, including a panel of cell surface CD markers that may define the cellular phenotype for each cell types are identified. Interestingly, these cell subtypes respond quite differently in the presence of either FOLFOX or FOLFIRI treatment. We developed a drug resistant/response scoring index system based on documented cancer hallmarks and top networks. Interestingly, these transcriptome-based resistant/response index correlate very well with the patient treatment response. We thus have established an effective, quick and robust system for evaluating drug response in vitro.

Project 2: Kinase inhibition in metastatic colorectal carcinoma

This Project is led by Qiang Tian at ISB, working in collaboration with Shao-En Ong and Raymond Yeung at UW.

Specific Aim 1: Kinase identification in mCRC

Major Task 1: kinobead assay

As indicated in our last progress report, the kinobead assay has been used in a number of human primary liver cancers with success. Based on protein abundance, we were able to identify pathways that are up-regulated including those known to be involved in cancer biology such as cell cycle kinases, p53 downstream targets and DNA damage response (Fig. 2).

Major Task 2: Proteogenomic analyses

Based on the samples that were analyzed by RNAseq in Project 1, we plan to perform global and kinobead enriched phosphoproteomic analyses in parallel to further highlight those pathways and targets that are abnormal in colorectal cancers. This work is currently in progress.

Specific Aim 2: Test kinase inhibitors using TSC

Major Task 1: Kinase inhibition in TSC

We await the results from Aim 1 to select kinase inhibitors for evaluation.

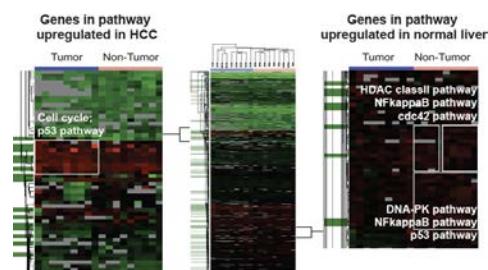


Figure 2. Differentially expressed genes cluster in relevant signaling pathways in HCC and non-tumor liver (NTL).

Major Task 2: Evaluate hepatotoxicity of inhibitors

In parallel with our tumor slice culture, we have also examined the adjacent non-tumor liver (NTL). It turns out that the liver slices are informative of an acute phase response normally observed in 'injured' livers in vivo. Such in vitro behavior can be exploited to study mechanisms of acute liver injury response as highlighted in our recent publication (1).

Project 3: Immune evasion in mCRC: Combination Immunotherapy

This project is under the direction of Venu Pillarisetty as PI, and Nick Crispe as co-PI, both at the University of Washington.

Specific Aim 1: Optimizing TIL function

Major Task 1: Define the expression of immunosuppressive molecules

Using our previously published method(2), we have performed the tumor slice culture *in vitro* experiments on a total of 16 mCRC tumors. We have spent the past year optimizing a 6-color multiplexed immunohistochemistry (mIHC) panel and successfully used in original tumor slides, unfortunately, there were too much background on tissue slices we tested. We are now ready to test the optimal conditions of mIHC to work on our tissue slice culture or using alternative methods to detect immunosuppressive signals within the colorectal cancer liver metastasis tumor microenvironment. We have obtained the anti-CEA CAR-T cells to be used in the slice culture system (See Specific Aim 2).

Major Task 2: Effects of checkpoint inhibition on TILs

We have created TSC from a total of 16 mCRC tumors during this project period and found that used PD-1 blocking antibody in conjunction with AMD3100 (a CXCR4 blocking small molecule) induced a synergistic effect on tumor cell killing, measured via counting cleaved caspase 3⁺ cells in slices compared to controls in some of the mCRC samples. More surprising, IL10 blockade accelerated cell apoptosis compared to control group in all mCRC treated cases. Cytokine levels in the culture supernatant we tested (4 cases) confirmed that immune cytotoxicity (such as GM-CSF, TNF alpha, IL2, and IL8) appear to be increased after IL10 blockade in all treated tumors. In some of mCRC cases, IL10 blockade, as well as combination treatment of PD1 blockage with AMD3100, increased IFN gamma and Granzyme B level. These findings are the results of several replicate experiments and indicated that the increased cytotoxic effect from IL10 blockade appears to be T-cell mediated. We are working single cell RNA sequence technique to fully define the mechanism of action.

Specific Aim 2: Optimizing CAR-T cell function

Major Task 1: Defining the impact of immunosuppressive signals on CAR-T cell function

Subtask 1: Assessment of CAR-T cell viability and function in TSC

In determining the ability of CAR-T cells to function in the human tumor slice culture model, we utilized live confocal microscopy of the slices in culture conditions consisting of a humidified, temperature controlled CO₂ chamber. Following staining of both control CAR-T cells and anti-CEA CAR-T cells with CFSE, 1 x 10⁶ CAR-T cells were applied to the slices. After one day, we found CFSE-labelled CAR-T cells to be infiltrated amongst tumor cells and other cells within the tumor microenvironment within the slice. The function of CAR-T cells can be assessed with SR-FLICA, a reagent which produces a fluorescent signal in the presence of activated cleaved caspases 3 and 7, thereby indicating apoptosis of a cell. We found that after treatment with anti-CEA CAR-T cells, a greater percentage of tumor cells are undergoing apoptosis than in a slice treated with control CAR-T cells (Figure 3A, B).

Major Task 2: Optimizing CAR-T cell function in the tumor microenvironment

Given the findings of increased tumor cell death evidenced by cleaved caspase 3 IHC staining following anti-IL-10 treatment, we hypothesized that inhibition of the immunomodulatory IL-10 cytokine would synergize with anti-CEA CAR-T cells in causing CEA⁺ tumor cell death. We again utilized live microscopy to directly visualize the microenvironment of CRCLM in TSC. We found an increased proportion of EpCAM⁺ tumor cells are within close proximity (within 20 μ m) of CD8⁺ CAR-T cells after treatment with both anti-CEA CAR-T cells and anti-IL-10 antibody (Figure 3C). In addition, there is greater apoptosis exhibited in tumor cells with a CAR-T cell nearby, while no difference in the percentage of apoptosis cells was found in tumor cells with no nearby CD8⁺ cell9 (Figure 3D). This experiment was repeated in a 2 separate cases of a different human CRCLM tumor with similar results.

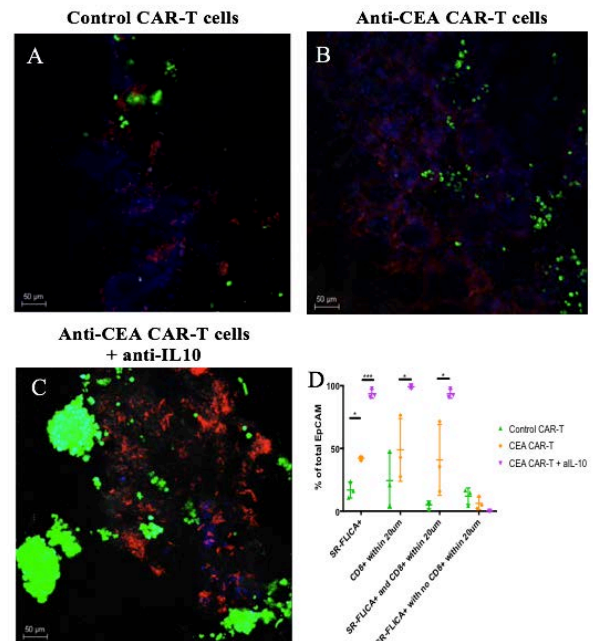


Figure 3 (A-C) Representative still images of confocal microscopy (20x) from human CRCLM slice culture on day one after treatment. EpCAM⁺ cells labelled red, CAR-T cells labelled green, and nuclei labelled blue. (D) Cell counts in one case of human CRCLM treated with CAR T cell therapy alone and with anti-IL-10 antibody treatment. Apoptotic cells, indicated by positivity for SR-FLICA, are in greatest proportion in a slice treated with combination anti-CEA CAR T and anti-IL-10 antibody. Similarly, CD8⁺ cells are found in close proximity more frequently in the slice with added anti-IL-10 antibody. The increased EpCAM⁺ cell apoptosis is due to an increase in apoptosis of the cells with nearby CD8⁺, as there is no difference amongst treatment in the tumor cell death when no CD8⁺ cell is nearby.

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

Nothing to report

How were the results disseminated to communities of interests?

Manuscripts for each of the Projects are currently under preparation.

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

Our progress is on schedule except for Project 2 (phosphoproteomics). We will devote significant effort to complete this Aim in the next reporting period. All of the reagents and tools are in place to perform the experiments.

4. IMPACT:

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

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS

No significant problems encountered.

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

N/A

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on this project?

Institute for Systems Biology:

Name:	Qiang Tian
Project Role:	Principal Investigator
Research Identifier:	
Nearest person month:	1
Contribution to project:	Dr. Tian directs the overall ISB efforts to ensure smooth integration and analysis of genome and transcriptome data generated from the same CRC patient cohort from his NCI-funded R01 project and kinome data from the current project. He supervises the bioinformatics scientist and communicates with partnering collaborators at the University of Washington.

Funding support:	
Name:	Xiaowei Yan
Project Role:	Bioinformatics Scientist
Research Identifier:	
Nearest person month:	2
Contribution to project:	Dr. Yan is responsible for computational analysis of the genomic data and transcriptomic data of CRC tissues before and after drug treatment, and will integrate these with the kinome data generated from the Eng-Ong lab at University of Washington.
Funding support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

There are 3 projects that make up this Award, each with its own P.I., working closely together to maximize scientific engagement and productivity. The overall P.I., Raymond Yeung (University of Washington), is largely responsible for the work performed in Project 1 with the assistance of a Co-P.I., Qiang Tian. Project 2 is headed by Qiang Tian at the Institute for Systems Biology working closely with Shao-En Ong (University of Washington). Venu Pillarisetty (University of Washington) heads the third Project. Each of the P.I. and Co-P.I. crafted the progress reports for their respective Projects and are glued together as a single document.

9. APPENDICES

none

Reference

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