

AWARD NUMBER: W81XWH-14-1-0110

TITLE: A Molecular Framework for Understanding DCIS

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REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE October 2015		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2014 - 29 Sep 2015	
4. TITLE AND SUBTITLE A Molecular Framework for Understanding DCIS				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-14-1-0110	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Gregory Hannon PhD E-Mail: greg.hannon@cruk.cam.ac.uk				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) CRUK Cambridge Institute Li Ka Shing Centre Robinson Way Cambridge CB2 0RE UK				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Abstract This project centers on creating a molecular framework of DCIS (ductal carcinoma in situ). DCIS is considered to be the precursor to Invasive Ductal Carcinoma (IDC), the most common form of breast cancer. IDC accounts for 80% of all breast cancers, predominantly affecting women aged 55 and older; however, at least a third of women with IDC are diagnosed before they reach 55. Not all patients with DCIS will develop IDC however, we are looking for ways to better predict those patients that need life-saving treatment, and separate these from those patients who are less at risk. So far we have 2060 lesions dissected from freshly frozen biopsies, these have been annotated by our pathologist and prepared to be taken on for sequencing. The tissue includes DCIS, IDC, stroma adjacent to DCIS/IDC and normal tissue. We have initiated the RNA sequencing from these samples and also the DNA sequencing.					
15. SUBJECT TERMS DCIS, IDC, LCM, RNAseq, DNAseq, evolution					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	6	19b. TELEPHONE NUMBER (include area code)

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A Molecular Framework for understanding DCIS

Award No. W81XWH-14-1-0110

Annual report year 1

1

Introduction

This project centers on creating a molecular framework of DCIS (ductal carcinoma in situ). DCIS is considered to be the precursor to Invasive Ductal Carcinoma (IDC), the most common form of breast cancer. IDC accounts for 80% of all breast cancers, predominantly affecting women aged 55 and older; however, at least a third of women with IDC are diagnosed before they reach 55.

Utilizing a unique bank of frozen mammary biopsies, containing samples with DCIS alone, and a combination of DCIS and IDC, we aim to profile both DCIS and related tissue components. It is our aim to sample the ~300 biopsies, and compare both by RNA seq, and whole genome amplification, DCIS lesions, within, and between patients, and see how these may be correlated with IDC lesions. We also intend to look for changes in the stroma between those patients that present with IDC and those that do not. This work aims to identify characteristics that may be suggestive of a patients' likelihood of progressing from DCIS to IDC, with the purpose of reducing the need for over treatment for this disease.

2.

Body

Biopsies are analyzed in two ways: sections are cut over 5 slides, the first and last slide are sent to be annotated by our project pathologist. Lesions of DCIS, IDC, Stroma (both adjacent to DCIS/IDC and distant), immune infiltrates, and normal ducts are indicated. The remaining sections are dissected for the annotated regions using the Laser capture microscope. Three replicates (from adjacent sections) are processed for RNA sequencing, and a further replicate is processed for DNA sequencing. Approximately 100 sections are processed per sample. The libraries are sequenced and analyzed by the team of bioinformatics, including information such as % mapping, gene assignment and duplication %. Information relating to tissue type, tissue area, breast cancer subtype and patient information is then collated and combined with the sequencing data.

3.

Accomplishments

Major goals of the project (as stated in SOW)

1. Sample collection/annotation (Duke)
2. Laser capture of frozen material (CSHL/Cambridge)
3. Exome capture and DNA sequencing (CSHL/Cambridge)
4. RNAseq library construction (CSHL/Cambridge)
5. Analysis DNA data (CSHL/NYGC)
6. Analyze RNA differential expression (NYGC)
7. Analyze stroma compartments (CSHL/Cambridge)
8. Technical validation of potential markers (Cambridge)
9. Validate potential markers in FFPE cohort (Duke/Cambridge)
10. Validate in longitudinal cohort (Duke /Cambridge)
11. Nominate candidates for clinical validation (Duke /Cambridge)

What was accomplished under these goals

This year we have initiated goals 1-6. Due to a delay in Duke receiving the IRB for this project we have been working on samples that were sent under the pilot scheme in addition to further samples we have received towards this project. With these we have been working to improve pipelines and better understand the data that we are getting from the sequencing. In total we have processed 35 patient samples. These include 2060 lesions that have sectioned on the LCM post annotation by Joe Geradts (the breast pathologist). These include DCIS lesions, IDC, stroma adjacent to DCIS, stroma adjacent to IDC, atypia lesions, Stroma away from disease, normal ductal epithelium and immune infiltrates. We have sequenced 500 libraries with the Clontech RNA seq low input kit. During this time Clontech updated kits and we found that the % of rRNA was increasing (up to 20% in some libraries). We spent some time working on methods to

improve this and are now back down to ~2% rRNA. We have found there is varying degrees of % uniquely mapping/ % assigned to genes. With approximately 50% uniquely mapping and ~30% assigned to genes, some correlations could be seen between quality of library and patient sample, and also with tissue type, ie. Stroma tissue tends to perform less well.

We are working on methods to improve this, as once we investigated further, we found that the low input going into the library prep seemed to result in an increased presence of amplification primers, these caused the sequencing pass rate to be effected due to the low diversity at the start of the reads. The bioinformatics team at the New York Genome Center (NYGC), are currently using the data to look for correlations and clustering that could be associated with the current breast cancer subtypes.

We initiated DNA libraries using both the sigma single cell WGA kit and Nextera kit, with Nextera Exome capture, however we found the Exome capture to be unsuccessful due to the low coverage assigned to the probes. We are now working with the NYGC to run samples on the X10 sequencing machine (where library prep is included in the cost of the run). Due to the longer reads that the X10 provides we are modifying the protocol to remove the need to shear the libraries, by testing different enzymatic protocols to remove the adapters from the WGA to allow sequencing. We expect a pipe line for these DNA libraries to be established soon and we will use this to contribute to Aim 1. The Evolution of DCIS.

Opportunities for training and professional development

Nothing to report (not intended for training)

Results disseminated to communities of interest

Nothing to report

Plan for next reporting period

We intend to have Aim 1 fully under way, if not completed. With the construction of DNA libraries we plan be able to answer the question of whether DCIS is a clonal disease, and if IDC is an independent disease or is a progression from DCIS.

We plan to have more RNA libraries made that will hopefully be able to classify DCIS into subtypes, be that similar to IDC subtypes, or entirely different subtypes. We hope to have some idea about how these could be related to patient outcome, but will likely require the addition of further patients in the coming years to bring about more sound conclusions and any candidate markers.

4. Reportable outcomes

Nothing to report

Actual or anticipated problems or delays and plans to resolve

There was a substantial delay in Duke obtaining the IRB to send us samples. However I think this has been resolved and we were able to still continue with samples that were received under the pilot but will still contribute to this project. We also moved the lab from CSHL to Cambridge, however, while this move was being carried out we were still able to continue at CSHL until a suitable person at Cambridge was trained to assist in the project.

Changes in use or care of human subjects

Nothing to report

Products

Nothing to report

5. Participants & other collaborating organizations

Individuals worked on the project

Name : Greg Hannon

Project Role: Initiating PI – contributed to project design and liaising with bioinformatics team

Nearest person month worked: 1 CM (10% x 8 months)

Funding support: CR-UK and Royal society

Name : Clare Rebbeck

Project Role: Co-PI – contributed to project design, staining strategy, dissecting with the LCM, RNA and DNA library preparation and liaising with Bioinformatics team and pathologist.

Nearest person month worked: 12 CM

Name : Elena Rozhkova

Project Role: Post doc – contributed to dissecting with the LCM and RNA library preparation in the early stages while at CSHL

Nearest person month worked: 6 CM

Funding support: philanthropic support

Name : Jian Xian

Project Role: senior research assistant - contributed to dissecting with the LCM and RNA and DNA library preparation

Nearest person month worked: 8 CM

Funding support: CR-U

Name : Martin Fabry

Project Role: student – contributed to DNA library preparation

Nearest person month worked: 3 CM

Name : Sophie Watcham

Project Role: research technician - contributed to DNA library preparation

Nearest person month worked: 3 CM

Name : Meagan Keane - contributed staining and RNA and DNA library preparation

Project Role: research technician

Nearest person month worked: 4 CM

Change in active support since last report

Nothing to report (this is the first reporting period)

Other organizations involved as partners

Duke university – collaboration to provide tissue samples and clinical annotation; as detailed in the grant application.

New York Genome Center – Collaboration with the bioinformatics team to analysis the data; As detailed in the grant application

7. Conclusions

We have initiated both the RNA sequencing and the DNA sequencing and have laser microdissected 35 patients.