Schulich MEDICINE & DENTISTRY

Project Title

Assessment of novel genomic signatures to measure response to Paclitaxel and Gemcitabine treatment in breast cancer patients

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Project Description

Background: Chemotherapy is recommended to breast cancer (BC) patients with developed metastases, basal-like BC, and high-risk indications[1]. Metastatic breast cancer response rates to these agents are low, at 22% for Pac, 37% for Gem, and 41% for combined treatment with both agents[2]. The premise of this study is that genetic analysis of targets of these drugs and associated biochemical pathways may provide a more effective treatment strategy in BC, since patients expected to exhibit a poor response could be treated with different agents. We analyzed gene expression, copy number changes, and mutations in target genes in BC cell lines, and derived gene signatures that are associated with concentrations of Paclitaxel (Pac) and Gemcitabine (Gem) that inhibit 50% growth of different cell lines. A support vector machine (SVM) analysis of mutation, gene expression and copy number in 49 cell lines identified signatures of 15 genes in Pac pathways and 10 genes in Gem pathways with 80-85% accuracy. Response to Pac is based on gene expression leveles of *ABCC10, BCL2, BCL2L1, BIRC5, BMF, FGF2, FN1, MAP4, MAPT, NKFB2, SLCO1B3, TLR6, TMEM243, TWIST1*, and *CSAG2*. Gene expression values (of *ABCB1, ABCC10, CMPK1, DCTD, NME1, RRM1, RRM2B*) and copy numbers (of *ABCC10, NT5C, TYMS*) comprised the SVM for Gem response.

Hypothesis/Objective: We hypothesize that response to Pac and Gem in BC patient populations can be predicted with the same gene signatures obtained from analysis of BC cell lines.

Aims: Determine 1) gene expression differences of signature genes in tumour samples using real time, quantitative RT-PCR; 2) whether the derived SVM model can predict response to Pac or Gem in these tumour samples.

Proposed Methodology: Using FFPE tissue samples obtained from clinical collaborators at the Italian National Cancer Institute (including 31 primary tumours, 5 metastatic tumours, lymph node positive (2) and negative (13), and 10 normal blocks). Pac and Gem response of these patients is known. We will complete a blinded study to determine whether we can predict tumour response based on gene expression patterns of the relevant genes identified using BC cell lines. We are currently extracting DNA and RNA for gene expression and DNA mutation studies.

The student will work under the guidance of both Dr. Peter Rogan, as well as a senior PhD student who is working with these tissue samples. They will select and verify control (house keeping) genes as well as design primers for the real time RT-PCR experiment. The student will use the Cancer Genome Atlas breast cancer gene expression and RNA sequencing data to select control genes that are stable in expression in breast cancer tumours, and to design primers in exons for all genes that are not alternatively spliced. Our lab currently has all of the data downloaded, and the processed data is publically available online.

If qRT-PCR results are impacted by the quality of the RNA extracted from FFPE material, submit the samples and genes to the University Health Network Microarray Centre in Toronto for single molecule analysis using the Nanostring nCounter system. The nCounter system is a sensitive method for gene expression studies, and uses single molecule imaging to detect and count multiple targets without requiring any amplification.

Expected Outcomes: The summer student will aim to validate the previously established gene signature that was derived using breast cancer cell lines. In addition, the summer student will also look for new patterns arising from the tumour samples that relate to Gem and Pac resistance, but were not identified using cell lines.

1. Largillier, R. et al. Annals of Oncology 19, 2012-2019 (2008); 2. Amadori et al. Annals of Oncology 17:173-176 (2006).

Research Environment

Laboratory personnel include 4 graduate students from the Departments of Biochemistry (1 PhD,1 MSc), Pathology (1 PhD), and Computer Science(1 PhD), 2 staff research technologists, 2 professional software developers, and 4 undergraduates. The directors, Drs. Joan Knoll (Medical Leader, Molecular Diagnostics, London Health Sciences Center) and Peter Rogan are actively involved in day-to-day activities of all laboratory members.

The Rogan-Knoll laboratory is fully equipped for human genomic, expression, cytogenetic and bioinformatic analysis. The proposed study will utilize instruments to prepare and sequence genomic and RNASeq libraries (Illumina GAIIx), PCR and real time PCR (Eppendorf and BioRad instruments). We also possess a microarray DNA synthesizer (Combimatrix) to synthesize capture reagents for next generation sequencing. These reagents are used to enrich for sequences from genes and pathways whose proteins are targets of Gem and Pac. a microarray reader (GenePix) for copy number genomic analysis of these genes. Finally, we have developed a software pipeline for RNA and DNA sequence alignment, variant calling and mutation and differential expression analysis. The proprietary mutation analysis software has been developed by Cytognomix Inc and is available to members of the Rogan and Knoll laboratories. An academic version of this software also runs on a queue dedicated to our laboratory on a 128 CPU, high performance computing cluster hardware that we have contributed to SHARCNET.

Expected Objectives/Accomplishments for Student within 16 weeks

Using a gene signature and a support vector machine classifier that we developed to predict cell line response to Pac and Gem, the summer student will test a set of phenotyped BC tumour samples from patients treated with these drugs. The student will employ the statistical and machine learning methods, i.e. support vector machine learning (SVM) and multiple factor analysis (MFA) to reveal critical genes and pathogenetic mechanisms associated with drug resistance. Those studies revealed that target gene expression levels for both drugs, with gene copy number additionally for Gem could predict the concentration of drug that inhibited cell line growth.

During the first 4 weeks, the student will design RT-PCR oligonucleotide primers for target and control genes which span different exons. Normal control genes with high fidelity mRNA splicing in tumours will be selected by the student by sequence analysis of BC RNASeq data from the Cancer Genome Atlas. RNA has been previously extracted from the samples and from adjacent matched, normal breast tissues. Real time, quantitative RT-PCR studies will be performed during the subsequent 8 weeks. During the final 4 weeks, after normalization against control genes among different samples, s/he will determine the relative abundance of each target gene and input these results into the SVM to predict the response for each drug. The observed clinical response will be compared these predictions.

In addition to the gene expression study, a PhD student in the lab is sequencing the same genes in these tumours using a capture array that was developed in the lab. The summer student will have the

opportunity to aid in the gene capture and sequencing lab work, as well as contribute to the DNA sequence analysis.

The student will also gain experience in pre- and post-processing of sequences and analysis of high throughput expression data using our existing software pipeline. They will also perform the MFA and SVM using previously established methods and programs written for MATLAB and R languages.

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