Expression Analysis of Cancer Cell Lines Treated with Omeprazole

Hailey Caswell, Christopher Farrell, PhD[1].
Presbyterian College School of Pharmacy Presbyterian College School of Liberal Arts

Background

The American cancer society estimates 16,170 deaths in 2020 with 20% survival rate five years post esophageal cancer diagnosis[1]. The poor prognosis is connected to chemotherapy resistance within cancer cells. MDR1, also known as PGP, is a transport protein that utilizes ATP for active transport of cytotoxins out of the cell. Studies have shown that the initiation of chemotherapy causes MDR1 expression to increase, resulting in drug resistance[2]. It is suspected that PGP substrates will also alter the expression of MDR1 and result in the development of chemotherapy resistance in chemo-naïve cells. Esophageal cancer diagnoses are commonly accompanied by chronic acid reflux[3]. Reflux medications are PGP substrates. We explored the effects of the reflux drug Omeprazole (OM) on the transcriptome of esophageal cell lines OE19 and OE33 as well as colorectal cell line Caco2. Following sequence analysis, we expect to see increased induction of MDR1(ABCB1) and altered drug metabolizing expression across the treatment groups. We aim to be able to provide personalized treatment plans for cancer patients exposed to PGP substrates.

Methods

Cell Lines

Three cancer cell lines, two Esophageal and one Colorectal, were obtained. All three cell lines used are from an Adenocarcinoma, the most common form of esophageal cancer in the united states[3].
Cell line OE33 also expresses Barrett's Esophagus. Barrett's is damage and cellular change to the lining of the esophagus due to prolonged exposure to stomach acid. An esophagus inflicted with Barrett's appears thick and red. Gastroesophageal reflux disease (GERD) is the leading cause of Barrett's Esophagus, symptoms include acid reflux and heartburn. The damage to the esophagus in Barret's results in an increased risk of Esophageal cancer[4].

Cell Line Information

Cell line	Cancer type	Cell type	Growth Mode	Sex	Age
OE19	Esophageal adenocarcinoma	Epithelial	Adherent	Male	72
OE33	Esophageal adenocarcinoma	Epithelial	Adherent	Female	73
CACO2	Colorectal adenocarcinoma	Epithelial	Adherent	Male	73

Table 1: Basic information from the three utilized cell lines.
Displays the general details of the three cell lines. OE19 and 33 are esophageal and Caco2 is colorectal

Establishing Chemotherapy Resistance

The three cell lines were treated with Omeprazole for 14 weeks. Following treatment, treated cells and negative control (untreated) cells were harvested and total RNA was extracted using a Qiagen kit Samples were prepared into RNA-seq libraries using the TruSeq RNA sample prep kit and manufacturer protocol. We verified with QPCR that the ABCB1 (MDR1 gene) was induced.

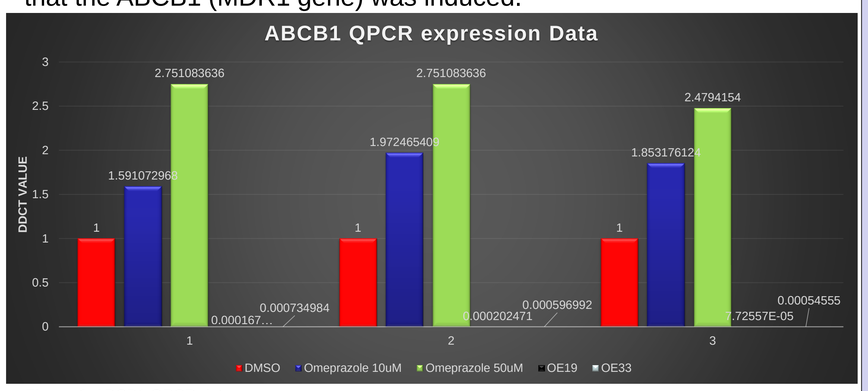

Figure 1: ABCB1 QPCR data:
Displays the induction of ABCB1 in cell lines treated with OM. Higher in colorectal lines that are used as a model DMSO = control 10uM and 50uM = colorectal

Methods

Establishing Chemotherapy Resistance

QPCR analysis of the colorectal cell line in treatment groups OM 10 uM and OM 50uM revealed that efflux pump ABCG2 was also induced.

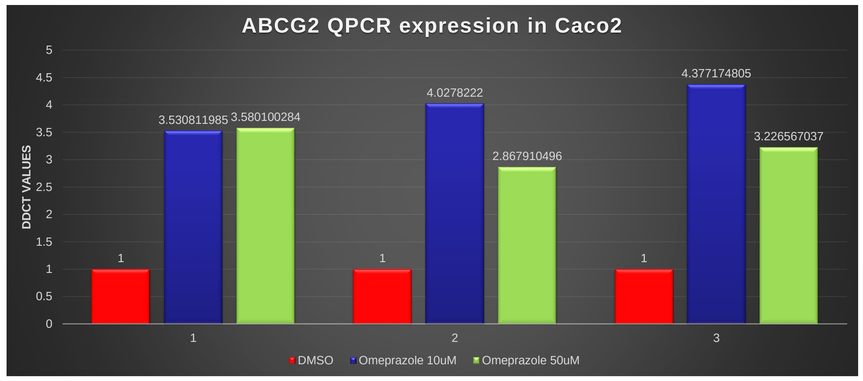

Figure 2: ABCG2 QPCR Data.
Displays the induction of Efflux pump aBCG2 in Caco2 treated cell line. DMSO = control

Data Analysis

Paired end cluster generation was preformed on the cBot following manufacture protocol. The clustered RNA-seq libraries were paired end sequenced with 2X125 cycles on a HiSeq2500. Fastq files were used for downstream analyses. The expression and sequence analysis was preformed in collaboration with Novagene. Using the CLC workbench Bioinformatics software at PC the data was analyzed for genomic variants and expressions in the transcriptome.

Expression Analysis

Drug Metabolizing Pathway

We identified CYP1A1 and CYP1B1 in the transcriptome of all three treated cell lines. CYP1A1 and CYP1B1 are metabolizing enzymes that have been shown to play a role in carcinogenesis and have been identified as upregulated in various cancers[5]. As expected, our data shows increased expression of both metabolizing enzymes across all treatment groups. Suggesting the ability of the PGP substrate drug to alter the expression of other pharmacokinetic proteins in the cell's drug pathway.

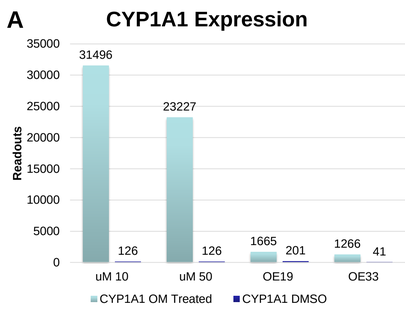

Figure 3a: CYP1A1 Expression
Increased expression in OM treated

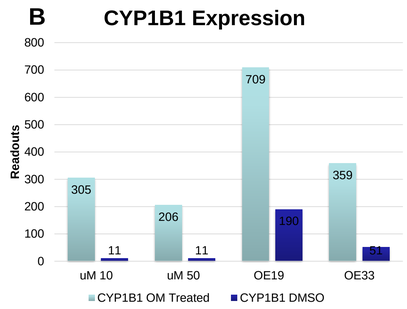

Figure 3b: CYP1B1 Expression
Increased expression in OM treated

Drug Resistance

ABCG2, like ABCB1, is an ATP dependent active transporter that exports toxins out of cells. It is a mark of chemotherapy resistance. The structure of the protein allows for a large range of substrate binding[6]. Originally noticed in the Caco2 QPCR, the expression data shows the efflux pump upregulated across all cell lines treated with the Omeprazole.

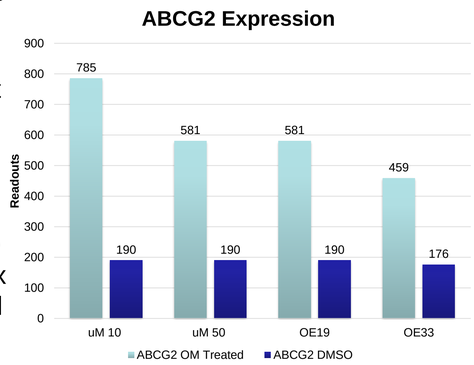

Figure 4: ABCG2 Expression.
ABCG2 efflux pump upregulated by OM

Expression Analysis

Prognosis

HSPH1 is a Nuclear exchange factor that promotes the release of ADP and prevents the denaturing of proteins under cellular stress[7]. HSPH1 is known to be upregulated in a variety of tumors[8]. HSPH1 is notably upregulated in the cell lines treated with omeprazole and suggests increased tumor aggression.

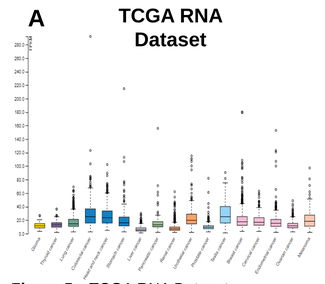

Figure 5a: TCGA RNA Dataset
Shows HSPH1 RNA in various cancer tissues. Low cancer specificity.
From: The Cancer Genome Atlas[9]

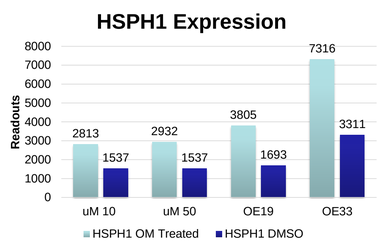

Figure 5b: HSPH1 Expression
Shows increased HSPH1 expression in OM treated cancer cell lines.

Potential Targets

CDK6 is involved in the control of the cell cycle and differentiation. Cancer cells alter the expression of CDK6 to disrupt the cell cycle and increase proliferation. Regulation of the gene through inhibitors may omit the effects of chemotherapy resistance.
YAP1 is involved in the regulation of CDK6 and has positively associated trends. YAP1 inhibitors could also be therapeutic[10].

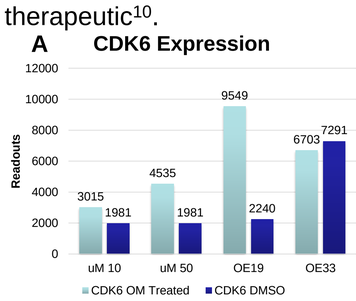

Figure 6a: CDK6 Expression
Expression increases significantly in OE19. Slightly decreases in OE33

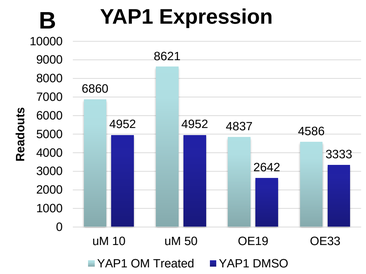

Figure 6b: YAP1 Expression
Expression increase across all OM treatment groups.

It was noted that cell line OE33, which expresses Barrett's Esophagus, showed slightly different trends in significance and expression. Patients that also display Barrett's may need varied treatment plans based off different expression.

Implications and Future Directions

The identification of chemo-resistant markers and the alteration of protein expression following Omeprazole treatment suggests the involvement of reflux drugs in the development of multidrug resistance in esophageal cancer. The recognition of this can provide personalized treatment guidelines for patients that have been prescribed reflux drug medications and have had prolonged PGP substrate exposure. Future research to expand on this topic includes further characterization of drug resistance in OM treated cells, confirmation of identified genes with QPCR, and a kinase assay to identify downstream activation of CDK6.

References

1. American Cancer Society. Cancer facts & figures. The Society, 2020.
2. Modulation of multidrug resistance: a paradigm for translational clinical research. Oncology (Williston Park) 1999; 13:183–7
3. The Mayo Clinic. Esophageal Cancer. 1998-2020 Mayo Foundation for Medical Education and Research (MFMER).
4. The Mayo Clinic. Barrett's Esophagus. 1998-2020 Mayo Foundation for Medical Education and Research (MFMER).
5. Androutsopoulos VP, Spyrou I, Ploumidis A, et al. Expression profile of CYP1A1 and CYP1B1 enzymes in colon and bladder tumors. PLoS One. 2013;8(12):e82487. Published 2013 Dec 16. doi:10.1371/journal.pone.0082487
6. Uniprot ID: Q9UNQ0 https://www.uniprot.org/uniprot/Q9UNQ0
7. Uniprot ID: Q92598 https://www.uniprot.org/uniprot/Q92598
8. Kai M, Nakatsura T, Egami H, Senju S, Nishimura Y, Ogawa M. Heat shock protein 105 is overexpressed in a variety of human tumors. Oncol Rep. 2003;10(6):1777-1782.
9. The Human Protein Atlas: HSPH1 https://www.proteinatlas.org/ENSG00000120694-HSPH1/pathology
10. Fan Li, Yan Xu, Bovey Liu, Pankaj Kumar Singh, et al. YAP1-Mediated CDK6 Activation Confers Radiation Resistance in Esophageal Cancer – Rationale for the Combination of YAP1 and CDK4/6 Inhibitors in Esophageal Cancer. Clin Cancer Res. 2019 (25) (7) 2264-2277; DOI: 10.1158/1078-0432.CCR-18-1029