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Celecoxib pre-treatment in human colorectal adenocarcinoma patients is associated with gene expression alterations suggestive of diminished cellular proliferation

James Todd Auman[a,e], *Robert Church*[b,e], *Soo-Youn Lee*[a], *Mark A. Watson*[c,d], *James W. Fleshman*[b,d], *Howard L. Mcleod*[a,*]

[a]*UNC Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina, Chapel Hill, Campus Box 7360, Room 3203 Kerr Hall, Chapel Hill, NC 27599-7360, United States*
[b]*Washington University, Division of Colorectal Surgery, School of Medicine, St. Louis, MO, United States*
[c]*Washington University, Department of Pathology, School of Medicine, St. Louis, MO, United States*
[d]*The Siteman Cancer Center, St. Louis, MO, United States*

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ABSTRACT

Cancer cells treated with the cyclooxygenase-2 inhibitor celecoxib show growth inhibition and induced apoptosis. This study was conducted to determine if the same processes are relevant to celecoxib's effects on human colorectal adenocarcinomas treated *in vivo*. A cohort of 23 patients with primary colorectal adenocarcinomas was randomised to receive a 7-d course of celecoxib (400 mg b.i.d.) or no drug prior to surgical resection. Gene expression profiling was performed on resected adenocarcinomas from the cohort of patients. Using fold change (>1.5) and *p*-value (<0.05) cut-offs, 190 genes were differentially expressed between adenocarcinomas from patients receiving celecoxib and those that did not. The celecoxib pre-treated samples showed decreased expression levels in multiple genes involved in cellular lipid and glutathione metabolism; changes associated with diminished cellular proliferation. Celecoxib pre-treatment for 7 d *in vivo* is associated with alterations in colorectal adenocarcinoma gene expression which are suggestive of diminished cellular proliferation.

1. Introduction

The inducible form of cyclooxygenase (COX), COX-2 is markedly up-regulated in pathological conditions such as inflammation and cancer.[1] For instance, a majority of colorectal adenocarcinomas display increased expression levels of the COX-2 protein.[2] Additionally, COX-2 expression levels have been associated with invasion depth,[3] liver metastasis[4] and poor clinical outcome.[5] Thus, the pharmacological inhibition of COX-2 activity has been explored as a therapeutic option for the management of colorectal cancer. In fact, administration of celecoxib, a selective COX-2 inhibitor, for 6 months resulted in a 28% reduction in the mean number of colorectal polyps in patients with familial adenomatous polyposis.[6] Furthermore, clinical trials of colorectal cancer patients are currently underway investigating the addition of celecoxib to conventional chemotherapeutic regimens.[7]

Investigations into celecoxib's mechanism of action have shown that *in vitro* administration leads to growth arrest and induction of apoptosis in a variety of cancer cells.[8–12]

* *Corresponding author:* Tel.: +1 919 966 0512/5242; fax: +1 919 962 0644.
E-mail address: hmcleod@unc.edu (H.L. Mcleod).
[e] These authors contributed equally to this work.
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Interestingly, celecoxib's anti-cancer effects appear to be independent of COX-2 inhibition, involving the inhibition of cell cycle progression, induction of apoptosis and inhibition of angiogenesis (reviewed in [13]). Investigations into celecoxib's mechanisms of action *in vivo* have also been conducted. Gene expression analysis of healthy colon tissue from patients with hereditary non-polyposis colon cancer (HNPCC) receiving celecoxib for 12 months displayed gene expression changes, suggestive of alterations in immune response, cell adhesion and transforming growth factor-β signalling.[14]

However, the mechanism with which celecoxib exerts its chemotherapeutics effects in colorectal tumours *in vivo* is currently not known. We conducted a Phase 0 study to examine the effects of celecoxib pre-treatment on primary colorectal adenocarcinoma gene expression to gain insight into the mechanisms underlying celecoxib's effects *in vivo*. Our results indicate that celecoxib pre-treatment *in vivo* is associated with multiple gene expression changes consistent with alterations in cellular lipid metabolism, glutathione metabolism and cell adhesion, in addition to differential expression of select genes involved in the immune response, apoptosis and angiogenesis. These results suggest that celecoxib tips the balance away from cellular proliferation towards growth inhibition in colorectal adenocarcinoma cells.

2. Materials and methods

Patients undergoing surgical resection of histologically proven primary colorectal adenocarcinomas were consented for participation in the study, which was approved by the Washington University, School of Medicine Institutional Review Board (Table 1). The patients enroled in this study were randomised to receive either 400 mg celecoxib two times per day or no COX-2 inhibitor for 7 d prior to surgical resection. This dose of celecoxib (400 mg b.i.d.) was chosen because it elicited a significant decrease in the mean number of colorectal polyps in patients with familial adenomatous polyposis.[6] A 7-d treatment regimen allowed the pharmacological evaluation to be performed without disrupting the surgical management of the colorectal adenocarcinomas. Patients in both groups were not allowed to take aspirin or other non-steroidal anti-inflammatory drugs (NSAIDS) before surgical resection to be eligible to participate in this study. Patients had received no prior therapy for their cancer. There were no apparent differences in either tumour cellularity or histology of the resection samples in the two groups.

Total RNA was isolated from surgically resected, histologically confirmed colorectal primary adenocarcinomas using a TRIzol RNA isolation kit (Invitrogen, Carlsbad, CA) according to the manufacturer's recommended protocol at the Siteman Cancer Center Tissue Procurement Core (St. Louis, MO). Total RNA (5 μg) from each sample was converted to double stranded cDNA using a dT-T7 promoter primer. The double stranded cDNA was then used as a template to synthesise biotinylated RNA, which was fragmented and hybridised to the Affymetrix HG_U95av2 microarray chip (Santa Clara, CA) using Affymetrix's labelling and hybridisation protocol within the Siteman Cancer Center Multiplex Gene Expression Core (St. Louis, MO).

Table 1 – Patient demographics

	Celecoxib	No drug
Gender		
Male	6	4
Female	5	8
Tumor site		
Ascending colon	3	2
Cecum	3	4
Descending colon	1	0
Hepatic flexure	1	1
Recto-sigmoid colon	0	2
Sigmoid colon	3	3
Clinical stage		
I	2	2
II	3	6
III	2	2
IV	4	2
Pathological grade		
Poorly differentiated	2	2
Moderately differentiated	7	9
Well differentiated	2	1

The array data were imported into GeneSpring GX 7.3 (Santa Clara, CA) and normalised using GC-RMA. Genes with normalised expression standard deviations <0.04 across all samples, essentially unchanging genes, were excluded from further analysis. Unsupervised, hierarchical clustering was performed on all samples and all changing genes (n = 10,083 genes) using uncentred correlation as the similarity matrix with complete linkage clustering was performed with Eisen's clustering programme.[15] Using a volcano plot in GeneSpring, 190 genes were identified with an absolute fold change greater than 1.5 and non-parametric p-value < 0.05 between adenocarcinomas from patients receiving celecoxib pre-treatment and adenocarcinomas from patients receiving no drug. Hierarchical clustering[15] was performed on all samples using the 190 differentially expressed genes identified in the volcano plot, using uncentred correlation as the similarity matrix with complete linkage clustering. The resulting heatmap and dendrogram were visualised using Java TreeView.[16] Gene expression enrichment analysis was performed using the analytical tools available at the database for annotation, visualization, and integrated discovery (DAVID) website: http://david.abcc.ncifcrf.gov/.[17] The data discussed in this publication have been deposited in NCBIs Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE11237.

3. Results

Unsupervised hierarchical clustering using all genes with differing levels of expression across all samples (n = 10,083 genes) showed no obvious separations based on gender, tumour site, clinical stage or celecoxib pre-treatment status (Fig. 1). Differentially expressed genes associated with celecoxib pre-treatment were identified by comparing gene expression intensities in celecoxib-treated patients and patients receiving no drug using a volcano plot. Using a >1.5