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Perturbations in Endocytotic and Apoptotic Pathways Are Associated With Chemotherapy-Induced Nausea

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Abstract

Background: While vomiting is well controlled with current antiemetic regimens, unrelieved chemotherapy-induced nausea (CIN) is a significant clinical problem. Perturbations in endocytotic and apoptotic pathways in the gut can influence the functioning of the microbiome-gut-brain-axis and the occurrence of gastrointestinal (GI) symptoms. However, limited information is available on the mechanisms that underlie unrelieved CIN. **Objectives:** The purpose of this study was to evaluate for perturbed biological pathways associated with endocytosis and apoptosis in oncology patients who did (n = 353) and did not (n = 275) report CIN prior to their second or third cycle of chemotherapy (CTX). **Methods:** Oncology patients (n = 735) completed study questionnaires in the week prior to their second or third cycle of CTX. CIN occurrence was evaluated using the Memorial Symptom Assessment Scale. Pathway impact analyses (PIA) were performed in 2 independent samples using RNA-sequencing (sample 1, n = 334) and microarray (sample 2, n = 294) methodologies. Fisher's combined probability method was used to identify signaling pathways related to endocytotic and apoptotic mechanisms that were significantly perturbed between the 2 nausea groups across both samples. **Results:** CIN was reported by 63.6% of the patients in sample 1 and 48.9% of the patients in sample 2. Across the 2 samples, PIA identified 4 perturbed pathways that are involved in endocytosis (i.e., endocytosis, regulation of actin cytoskeleton) and apoptosis (i.e., apoptosis, PI3K/Akt signaling). **Conclusions:** Our findings suggest that CTX-induced inflammation of the GI mucosa, that results in the initiation of endocytotic and apoptotic processes in the gut, is associated with the occurrence of CIN.

Keywords

chemotherapy, nausea, gene expression, endocytosis, apoptosis

Chemotherapy-induced nausea (CIN) is one of the most feared and debilitating side effects of chemotherapy (CTX; Kuchuk et al., 2013). While antiemetic prophylaxis controls vomiting, persistent CIN remains a significant clinical problem (Roila et al., 2016). In our recent study (Singh, Kober, et al., 2018), 48% of the patients reported CIN prior to their second or third cycle of CTX. Findings from 2 studies suggest that CIN could be the sentinel symptom for the occurrence and severity of a wide array of CTX-induced symptoms (Donovan et al., 2016; Papachristou et al., 2019). Little is known about the molecular mechanisms that are associated with occurrence and/or severity of CIN. Findings from a recent review of candidate gene studies noted that the majority of the genes that were selected based on 3 hypothesized mechanisms for CIN (i.e., alterations in serotonin receptor, drug metabolism, and/or drug transport pathways) were not associated with either its occurrence or severity (Singh, Dhruva, et al., 2018).

An increased understanding of the mechanisms that underlie CIN may guide the development of targeted interventions for this persistent symptom that does not respond to

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evidence-based antiemetic regimens. Emerging evidence suggests that CTX-induced alterations in the functioning of the microbiome-gut-brain-axis (MGBA) are associated with the occurrence of CIN (Donovan et al., 2016; Singh, Dhruva, et al., 2020). Specifically, in our previous gene expression (GE) study (Singh, Dhruva, et al., 2020), perturbations in pathways involved in mucosal inflammation and disruption of the gut microbiome, that are known to effect the functioning of the MGBA, were associated with the occurrence of CIN. In terms of mucosal inflammation, the perturbed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways included those involved in cytokine-cytokine receptor interactions, mitogen-activated protein kinase activity, nuclear factor kappa beta (NF-κB) signaling, and chemokine signaling. In addition, the KEGG pathways that suggested that CIN occurrence was associated with disruptions in the MGBA included: intestinal immune network for immunoglobulin A production, NF-κB signaling, peroxisome-proliferation-activated receptor signaling, interleukin (IL)-17 producing helper T cell differentiation, tight junctions, and antigen processing and presentation. Taken together with several lines of preclinical (Logan et al., 2007) and clinical (Donovan et al., 2016; Keefe et al., 2004) evidence that suggest that CTX-induced activation of the MGBA may result in a variety of gastrointestinal (GI) symptoms (e.g., abdominal bloating), we suggested that CTX-induced mucosal inflammation and disruption of the gut microbiome can alter the bidirectional communication within the MGBA (Bajic et al., 2018) and result in the development of CIN and other GI symptoms reported by oncology patients receiving CTX.

A growing body of evidence suggests that perturbations in endocytotic (Lechuga & Ivanov, 2017) and apoptotic (Carneiro-Filho et al., 2004; Gibson et al., 2005; Keefe et al., 1997; Logan et al., 2007; Sonis et al., 2004) pathways in the gut can influence the functioning of the MGBA and the occurrence of GI symptoms. Endocytosis is a fundamental biological process that carries out essential cellular functions in epithelial cells. Within the GI system, enterocytes are regulated by endocytotic signals that result in: digestion and absorption of nutrients and drugs; barrier permeability to microorganisms, toxins, and antigens; and transcytotic crosstalk between the intestinal lumen and the lamina propria cells that have access to the circulation (Zimmer et al., 2016). While not studied in association with the administration of CTX, findings from a number of studies, in a variety of inflammatory bowel diseases, suggest that intestinal inflammation is an "upstream event" that results in alterations in endocytotic processes in enterocytes that are associated with disruptions in the functioning of the MGBA (Lechuga & Ivanov, 2017; Zimmer et al., 2016). Given that the administration of CTX produces intestinal inflammation and increases in gut permeability (Montassier et al., 2015), it is reasonable to hypothesize that the administration of CTX results in perturbations in endocytotic pathways that may be associated with occurrence of CIN.

Within the GI tract, apoptosis, a form of programmed cell death, is a fundamental mechanism that contributes to homeostasis by maintaining a strict equilibrium between cell proliferation in intestinal crypts and cell shedding from villus tips (Negroni et al., 2015). A growing body of both pre-clinical (Al-Dasooqi et al., 2011; Bowen, Gibson, Cummins, et al., 2007; Bowen et al., 2005, 2010; Gibson et al., 2005; Papaconstantinou et al., 2001) and clinical (Bowen et al., 2005; Keefe et al., 2000) evidence suggests that following the administration of CTX, small intestinal crypts undergo apoptosis that results in GI mucositis.

Given the limited amount of clinical research on associations between CTX-induced alterations in endocytotic and/or apoptotic processes and GI symptoms, including CIN, in the current study, we extend the findings from our previous GE study (Singh, Dhruva, et al., 2020) and evaluated for perturbed biological pathways associated with endocytosis and apoptosis in oncology patients who did and did not report CIN prior to their second or third cycle of CTX.

Methods

Patients and Settings

This study is part of a larger, longitudinal study, of the symptom experience of oncology outpatients receiving CTX whose details are published elsewhere (Miaskowski et al., 2014; Singh, Kober, et al., 2020; Singh, Paul, et al., 2020; Singh, Dhruva, et al., 2020; Singh, Kober, et al., 2018; Wright et al., 2015). Eligible patients were $\geq$ 18 years of age; had a diagnosis of breast, GI, gynecological (GYN), or lung cancer; had received CTX within the preceding 4 weeks; were scheduled to receive at least two additional cycles of CTX; were able to read, write, and understand English; and gave written informed consent. Patients were recruited from two Comprehensive Cancer Centers, a Veteran's Affairs hospital, and four community-based oncology programs.

Study Procedures

As described previously (Singh, Kober, et al., 2020), the study was approved by the Institutional Review Board at each of the study sites. Of the 2,234 patients approached, 1,343 consented to participate (60.1% response rate). The major reason for refusal was being overwhelmed with their cancer treatment. Eligible patients were approached in the infusion unit during their first or second cycle of CTX by a member of the research team to discuss study participation and obtain written informed consent. Data from the enrollment assessment (i.e., the assessment of nausea in the week prior to the patient's second or third cycle of CTX) were used in this analysis to create the nausea groups. Blood for ribonucleic acid (RNA) isolation was collected at the enrollment assessment. Medical records were reviewed for disease and treatment information. For this paper, a total of 735 patients provided blood samples for the GE analyses (see Supplementary Figure 1).

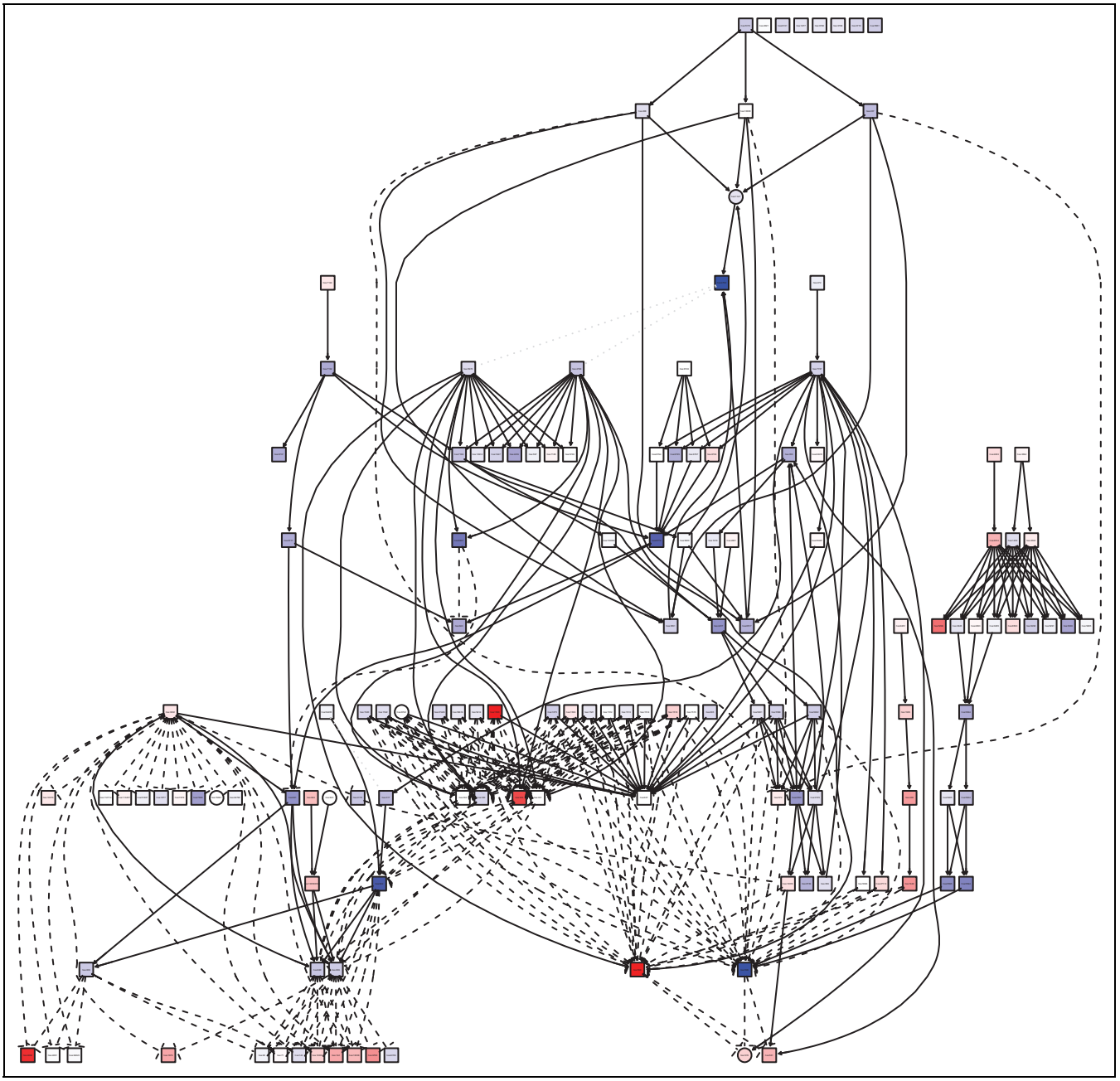

Figure 1. Graph summary of the perturbation propagation on the apoptosis KEGG signaling pathway (hsa04210) for Patients in Sample 2 (i.e., having Gene Expression Measured by Microarray). The Square Nodes Denote Genes with Gene Expression Changes and the Circle Nodes Denote All Other Nodes. The color of each node represents the perturbation (Red = Positive, Blue = Negative) and the Shade represents the strength of the perturbation. Note that the Square Nodes with No Parents will have no Accumulation. Colored version of this figure is available online.

Instruments

Demographic and clinical characteristics. Patients completed a demographic questionnaire, the Karnofsky Performance Status (KPS) scale (Karnofsky, 1977), and the Self-Administered Comorbidity Questionnaire (SCQ; Sangha et al., 2003).

Nausea assessment. The Memorial Symptom Assessment Scale (MSAS) was used to assess nausea as reported elsewhere (Singh, Dhruva, et al., 2020). Briefly, patients' reports of the occurrence of nausea were used to dichotomize the sample (Portenoy et al., 1994). Patients who provided a rating for occurrence, frequency, severity, and/or distress for the nausea item were coded as having nausea. Patients who indicated "no" to the occurrence item were coded as not having nausea.

Coding of the Emetogenicity of the CTX Regimens

Using the Multinational Association for Supportive Care in Cancer (MASCC) guidelines (Roila et al., 2016), each CTX

Table 1. Perturbed and Endocytosis and Apopotosis Related KEGG Pathways Between Oncology Patients With and Without Chemotherapy-Induced Nausea.

Pathway ID	Pathway Name	Adjusted Global pPERT
	Endocytosis	
hsa04144	Endocytosis	0.00084
hsa04810	Regulation of actin cytoskeleton	0.00785
	Apoptosis	
hsa04210	Apoptosis	0.00851
hsa04151	PI3K/Akt signaling pathway	0.00584

Note. KEGG = Kyoto Encyclopedia of Genes and Genomes; FWER = family-wise error rate; PI3K-Akt = phosphatidylinositol kinase-protein kinase B; pPERT = Perturbation *p*-value; RNA-seq = Ribonucleic acid sequencing.

drug in the regimen was classified as having minimal, low, moderate, or high emetogenic potential. The emetogenicity of the regimen was categorized into three groups (i.e., low/minimal, moderate, or high) based on the CTX drug with the highest emetogenic potential.

Coding of the Antiemetic Regimens

Each antiemetic was coded as a neurokinin-1 (NK-1) receptor antagonist, a serotonin receptor antagonist, a dopamine receptor antagonist, anti-psychotic, anti-anxiety, or a steroid. The antiemetic regimens were coded into four groups: none (i.e., no antiemetics administered); steroid alone or serotonin receptor antagonist alone; serotonin receptor antagonist and steroid; or NK-1 receptor antagonist and two other antiemetics (e.g., a serotonin receptor antagonist, dopamine receptor antagonist, prochlorperazine, lorazepam, and/or a steroid).

Acquisition and Processing of Gene Expression Data

The methods used for the GE analyses are described in detail elsewhere (Singh, Dhruva, et al., 2020). In brief, GE of total RNA isolated from peripheral blood of the 735 patients who provided a blood sample was quantified for 375 patients using RNA-sequencing (RNA-seq) (i.e., sample 1) and for 360 patients using microarray (i.e., sample 2). After quality control, the final dataset evaluated for GE for 334 patients in sample 1 and 294 in sample 2 (Supplementary Figure 1).

Data Analyses

Demographic and clinical data. Demographic and clinical data from the two patient samples were analyzed separately using SPSS Version 23 (IBM, Armonk, NY) and are described in detail elsewhere (Singh, Dhruva, et al., 2020). Univariate and multiple logistic regression analyses were used to determine significant covariates for inclusion in the differential expression (DE) analysis.

Pathway impact analysis. DE was quantified using generalized linear models separately for both samples (Singh, Dhruva, et al., 2020). These analyses were adjusted for demographic and clinical characteristics that differed between patients who did and did not have nausea. In addition, the models included surrogate variables generated by surrogate variable analysis (SVA) (Leek & Storey, 2007) using the sva Bioconductor/R package (https://bioconductor.org/packages/release/bioc/html/sva.html) to adjust for potential batch effects. For sample 1, three characteristics (i.e., KPS score, CTX cycle length, type of prior cancer treatment) and two surrogate variables were retained in the final model. For sample 2, four characteristics (i.e., having childcare responsibilities, KPS score, emetogenicity of the CTX regimen, cancer diagnosis) and 23 surrogate variables were retained in the final model. The DE results were summarized as the log fold change and *p*-value for each gene.

As previously reported (Singh, Dhruva, et al., 2020), to evaluate these results and interpret them in the context of apoptotic and endocytotic processes, we used pathway impact analysis (PIA) to test for patterns in higher orders of biology. PIA includes potentially important biological factors (e.g., gene-gene interactions, flow signals in a pathway, pathway topologies), the magnitude (i.e., log fold-change), and *p*-values from the DE analysis (reviewed in Mitrea et al., 2013). The PIA included the results of the DE analysis for all genes (i.e., cutoff free) to determine probability of pathway perturbations (pPERT) using Pathway Express (Draghici et al., 2007). A total of 208 signaling pathways were defined using the KEGG database (Aoki-Kinoshita & Kanehisa, 2007). Sequence loci data were annotated with Entrez gene identifier. The gene symbols were annotated using the HUGO Gene Nomenclature Committee resource database (Gray et al., 2013).

Fisher's combined probability test was used to combine the DE tests from both datasets using the uncorrected *p*-values (Fisher, 1925, 1948). The two datasets (i.e., sample 1 and sample 2) were merged at the gene level using the Entrez gene identifier. Significance of the combined transcriptome-wide PIA analysis was assessed using a family wise error rate (FWER) of 1% under the Bonferroni method (Draghici et al., 2007). Finally, we evaluated these results specifically for pathways in the context of apoptotic and endocytotic processes.

Results

Differences in Demographic and Clinical Characteristics

As previously reported (Supplementary Tables 1–3) (Singh, Dhruva, et al., 2020), after the multiple logistic regression analyses, patients in sample 1 who had a lower KPS score were more likely to be in the nausea group. Compared to patients who received CTX on a 14 day cycle, patients who received CTX on a 21-day cycle were less likely to be in the nausea group. Compared to patients who received only surgery, CTX, or RT, patients who received surgery and CTX, or surgery and RT, or CTX and RT were less likely to be in the nausea group. Patients in sample 2 who had childcare responsibilities and a

lower KPS score were more likely to be in the nausea group. Compared to patients who received a CTX regimen with minimal or low emetogenicity, patients who received a CTX regimen with high emetogenicity were more likely to be in the nausea group. Compared to patients who had lung cancer, patients who had GI cancer were 5 times more likely to be in the nausea group. Compared to patients who had GI cancer, patients who had GYN cancer were less likely to be in the nausea group.

PIA of the Whole Transcriptome

Annotation with ENTREZ gene identifiers was performed for both sample 1 and sample 2 (n = 11,577 and n = 20,216, respectively). Fold changes and *p*-values from the DE analyses for these genes were included in the PIA of the 208 KEGG signaling pathways. In the combined analysis using Fisher's combined probability method (Fisher, 1925, 1948), as previously reported (Singh, Dhruva, et al., 2020), the combined PIA analysis identified 37 KEGG signaling pathways that were significantly different between the nausea groups after correcting for multiple hypothesis testing using a strict FWER of 1% (adjusted global perturbation $p < 0.01$). Four KEGG signaling pathways were associated with mechanisms involved in endocytosis and apoptosis (Table 1; Figure 1).

Discussion

This study is the first to provide preliminary evidence that perturbations in both endocytotic and apoptotic processes are associated with the occurrence of CIN. These results extend the findings from our previous report (Singh, Dhruva, et al., 2020) that described associations with the occurrence of CIN and perturbations in 10 pathways involved in mucosal inflammation (four pathways) and disruption of the gut microbiome (six pathways). The identification of associations between endocytotic and apoptotic pathways and CIN occurrence are supported by the fact that GI intestinal inflammation from a variety of causes, including the administration of CTX, is associated with changes in both endocytosis (Lechuga & Ivanov, 2017) and apoptosis (Blander, 2016; Ruder et al., 2019).

While previous studies reported associations between CTX-induced apoptosis and a number of GI symptoms (e.g., mucositis, diarrhea; Keefe et al., 2000; Logan et al., 2007; Sonis et al., 2004), no studies were identified that found associations between either endocytosis and apoptosis and the occurrence of CIN. A number of mechanisms are involved in endocytotic (Lechuga & Ivanov, 2017) and apoptotic (Nunes et al., 2014; Sui et al., 2014) processes in the gut. We frame our discussion based on the specific biological pathways associated with endocytotic and apoptotic processes identified in this study and hypothesize how these two mechanisms are associated with the occurrence of CIN.

Endocytosis

Endocytosis and regulation of the actin cytoskeleton were the two KEGG pathways that were identified as perturbed endocytotic pathways (Table 1). While changes in the actin cytoskeleton have been associated with CTX-induced peripheral neuropathy (Kober et al., 2019; Malacrida et al., 2019), no studies were found that evaluated for associations between CTX-induced changes in the functioning of intestinal epithelial cells (IEC) and perturbations in pathways involved in endocytosis and regulation of the actin cytoskeleton.

Most of the research on endocytotic processes associated with the GI tract have focused on irritable bowel disease (IBD), Crohn's disease, and celiac disease (Lechuga & Ivanov, 2017; Zimmer et al., 2016). Most of these studies evaluated for changes in the permeability of the intestinal epithelial barrier associated with inflammation. Integral to the maintenance of the intestinal barrier are several multiprotein adhesive complexes, including apical tight junctions (Shen et al., 2011). Evidence suggests that the release of proinflammatory cytokines (e.g., tumor necrosis factor alpha (TNF-α), interferon gamma (INF-γ) increases endocytosis and mediates decreases in the expression of epithelial tight junction proteins (Capaldo & Nusrat, 2009; Ivanov & Naydenov, 2013; Onyiah & Colgan, 2016). CTX damages the epithelial cells of the entire alimentary tract which results in mucosal inflammation (Logan et al., 2007). Our previous findings support that perturbations in pathways involved in mucosal inflammation (e.g., cytokine-cytokine receptor interaction, nuclear factor kappa-beta (NF-κB) signaling pathway), as well as the tight junction pathway are associated with the occurrence of CIN (Singh, Dhruva, et al., 2020). Therefore, it is reasonable to hypothesize that alterations in endocytotic pathways are associated with the occurrence of CIN.

The association of apical tight junctions with the cortical actin cytoskeleton is essential to maintain the integrity and plasticity of the gut barrier (Ivanov et al., 2010; Shen et al., 2011). Disruption of the actin cytoskeleton was observed in IECs exposed to a variety of inflammatory mediators (Musch et al., 2006; Utech et al., 2005) as well as in tissue samples of patients with mucosal inflammation (Ivanov et al., 2010). While CTX drugs like paclitaxel are known to disrupt the functioning of the cytoskeleton of cancer cells and produce severe nausea, whether they have direct effects on the cytoskeleton of IECs warrants investigation.

Apoptosis

Compared to endocytosis, as early as 1983, findings from preclinical studies demonstrated that a variety of CTX drugs (e.g., doxorubicin, bleomycin, actinomysin D, cyclophosphamide) induced apoptosis at different cellular positions in intestinal crypt cells that was associated with differing degrees of mucosal injury (Ijiri & Potten, 1983, 1987). Subsequently, pre-clinical research focused on an evaluation of the apoptotic

mechanisms associated with the administration of methotrexate (Bowen et al., 2005; Gibson et al., 2005; Papaconstantinou et al., 2001), irinotecan (Al-Dasooqi et al., 2011; Bowen, Gibson, Stringer, et al., 2007; Gibson et al., 2003, 2007; Mayo et al., 2017), and 5-fluorouracil (5-FU) (Bach et al., 2006; Gao et al., 2014; Han et al., 2011). In terms of clinical research, in a study of 23 patients with heterogeneous cancer diagnoses and CTX regimens who underwent upper GI endoscopy with duodenal biopsy (Keefe et al., 2000), apoptosis increased sevenfold in intestinal crypts 1 day after the administration of CTX. In another randomized clinical trial of parenteral glutamine in patients with metastatic colon cancer who received 5-FU (n = 24; Decker-Baumann et al., 1999), while apoptosis was not evaluated specifically, patients who received glutamine had a significant reduction in mucositis and ulcerations of the gastric and duodenal mucosa. However, no between group differences were found in the incidence and severity in any adverse effects including nausea.

Under physiologic conditions, programmed apoptosis maintains the homeostatic balance of the GI mucosal. However, the administration of CTX damages IECs which causes the release of reactive oxygen species (ROS). As a result, the transcription factor NF-κB is activated which leads to the upregulation of several genes including those involved in the production of inflammatory cytokines, as well as adhesion molecules, and cyclo-oxygenase 2 (Bowen et al., 2006). An amplification cascade ensues that results in the transcription of genes that encode for mitogen-activated protein kinase (MAPK) signaling molecules. Activation of the NF-κB signaling and MAPK signaling pathways (Sonis, 2004), as well as continued synthesis and release of inflammatory cytokines, results in the loss of mucosal integrity along the GI tract (Logan et al., 2007; Sonis, 2004).

The NF-κB (Bowen et al., 2006; Tokuhira et al., 2015), MAPK (Gao et al., 2014; Osaki & Gama, 2013; Sui et al., 2014; Sun et al., 2015) and phosphatidylinositol 3′-kinase/Akt (PI3K/Akt; Pedersen et al., 2014; Tokuhira et al., 2015) signaling pathways are involved in the regulation of apoptosis (Mayo et al., 2017; Pedersen et al., 2014; Sui et al., 2014). Perturbations in both the NF-κB and MAPK pathways were associated with the occurrence of CIN in our previous study (Singh, Dhruva, et al., 2020). In terms of PI3K/Akt signaling, this pathway plays a pivotal role in apoptosis that is mediated by the generation of ROS (Dahan et al., 2008; Garcia et al., 2006), as well as in the regulation of the NF-κB signaling pathway (Tokuhira et al., 2015). While not studied in the context of CTX, the PI3K/Akt and MAPK signaling pathways were found to be activated in IEC cultures when they were exposed to lamina propria lymphocytes from patients with IBD. This finding suggests that IEC differentiation is accelerated in patients with IBD (Dahan et al., 2008). In our study, we found that perturbations in PI3K/Akt signaling pathway were associated with occurrence of CIN.

Four preclinical studies were identified that evaluated for changes in GE in the epithelial mucosa of the GI tract associated with the administration of doxorubicin (de Koning et al., 2007) and irinotecan (Bowen, Gibson, Cummins, et al., 2007; Bowen, Gibson, Tsykin, et al., 2007; Bowen et al., 2010). In addition, one study evaluated for changes in GE in the oral mucosa of patients with multiple myeloma who received high-dose melphelan (Marcussen et al., 2017) and from peripheral blood of patients with esophageal cancer prior to the receipt of 5-FU, cisplatin, and radiation therapy (Bowen et al., 2015). Consistent with findings from our previous (Singh, Dhruva, et al., 2020) and current study, perturbations were found in the following pathways associated with the administration of irinotecan: MAPK signaling (Bowen, Gibson, Tsykin, et al., 2007; Bowen et al., 2010), apoptosis (Bowen, Gibson, Tsykin, et al., 2007), cytokine-cytokine receptor interaction (Bowen, Gibson, Tsykin, et al., 2007), regulation of the actin cytoskeleton (Bowen, Gibson, Tsykin, et al., 2007), NF-κB signaling (Bowen, Gibson, Tsykin, et al., 2007), and PI3K/Akt signaling (Bowen, Gibson, Tsykin, et al., 2007). Neither of the human studies did a PIA. However, in a study that evaluated oral mucosa samples (n = 10; Marcussen et al., 2017), while genes associated with TNF pathways that favored anti-apoptotic effects were differentially expressed, their upregulation was independent of mucositis grade. In the study of patients with esophageal cancer (n = 31; Bowen et al., 2015), of the 84 immune genes investigated, TNF was significantly elevated (2.05-fold, $p = .025$) in the patients with more severe GI toxicity. Nausea and vomiting were the two symptoms with the highest severity ratings. Taken together, these preclinical and clinical findings suggest that perturbations in apoptotic pathways occur with the administration of CTX. Additional research is warranted to confirm their occurrence with CIN and other GI symptoms.

Limitations

While our study has numerous strengths including: a large sample size, stringent quality control procedures, the use of two complimentary methods to measure GE, strict criteria for DE and pathway perturbation selection, and the combination of results from independent tests across two samples, several limitations warrant consideration. While we have indirect evidence from blood samples to support our hypothesis that CTX-induced changes in endocytosis and apoptosis are associated with CIN and preliminary evidence of strong correlations between GE changes in the peripheral blood and small bowel biopsies of patients with celiac disease (Galatola et al., 2013), future studies are warranted that obtain tissue samples along the GI tract to provide direct evidence for this association. While our sample was large and representative of patients with CIN, our findings warrant confirmation in an independent cohort. Given that our phenotype and GE measures were done prior to the patients' second or third cycle of CTX, additional research is warranted to determine if these changes in GE and pathway perturbations occur at other time points during the administration of CTX and/or with the severity of CIN.

Conclusions and Directions for Future Research

Despite these limitations, our study is the first to report on associations between perturbations in endocytotic and apoptotic pathways and the occurrence of CIN. These findings are consistent with the growing body of evidence that suggests that GI inflammation in general, as well as CTX-induced inflammation of the GI mucosa, initiate endocytosis (Lechuga & Ivanov, 2017; Zimmer et al., 2016) and apoptosis (Ijiri & Potten, 1983, 1987). Findings from our previous (Singh, Dhruva, et al., 2020) and current study suggest that complex mechanisms underlie the occurrence of CIN and provide insights into why unrelieved CIN remains a significant clinical problem despite the use of evidence-based antiemetic guidelines. Future research needs to consider these mechanisms as potential targets for the development of new therapeutic agents.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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