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ARTICLE

Therapeutic Opportunities for Intestinal Angioectasia-Targeting PPARγ and Oxidative Stress

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Recurrent and acute bleeding from intestinal tract angioectasia (AEC) presents a major challenge for clinical intervention. Current treatments are empiric, with frequent poor clinical outcomes. Improvements in understanding the pathophysiology of these lesions will help guide treatment. Using data from the US Food and Drug Administration (FDA)'s Adverse Event Reporting System (FAERS), we analyzed 12 million patient reports to identify drugs inversely correlated with gastrointestinal bleeding and potentially limiting AEC severity. FAERS analysis revealed that drugs used in patients with diabetes and those targeting PPARγ-related mechanisms were associated with decreased AEC phenotypes ($P < 0.0001$). Electronic health records (EHRs) at University of Cincinnati Hospital were analyzed to validate FAERS analysis. EHR data showed a 5.6% decrease in risk of AEC and associated phenotypes in patients on PPARγ agonists. Murine knockout models of AEC phenotypes were used to construct a gene-regulatory network of candidate drug targets and pathways, which revealed that wound healing, vasculature development and regulation of oxidative stress were impacted in AEC pathophysiology. Human colonic tissue was examined for expression differences across key pathway proteins, PPARγ, HIF1α, VEGF, and TGFβ1. *In vitro* analysis of human AEC tissues showed lower expression of PPARγ and TGFβ1 compared with controls (0.55 ± 0.07 and 0.49 ± 0.05). National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) RNA-Seq data was analyzed to substantiate human tissue findings. This integrative discovery approach showing altered expression of key genes involved in oxidative stress and injury repair mechanisms presents novel insight into AEC etiology, which will improve targeted mechanistic studies and more optimal medical therapy for AEC.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ The clinical detection of angioectasia (AEC) has increased using push-enteroscopy, capsule enterography, colonoscopy, and esophagogastroduodenoscopy. Management is difficult. Currently, endoscopic ablation is an option for lesions within endoscopic reach, whereas angiogenesis inhibitors and octreotide are pharmacological agents additionally used in the treatment of AEC often with limited clinical benefit. The precise pathophysiology of AEC is unknown; however, AECs are known to result from an imbalance between the pro-angiogenic and anti-angiogenic factors.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ How do intestinal AEC develop and how can we design targeted therapeutic discovery for AEC.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Insight into the development of intestinal AEC and a targeted approach for novel therapeutic strategies.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ The results of this study demonstrate the complexity of AEC development and novel therapeutic directions that could impact patient care and treatment.

Angioectasia (AEC) lesions are common vascular abnormalities characterized by ectatic, dilated, and proliferated blood vessels, and are a significant source of obscure gastrointestinal (GI) bleeding. These aberrant blood vessels are typically < 10 mm in diameter, thin walled with little or no smooth muscle, malformed, and uncommunicative,[1,2] and symptomatically present with overt and occult GI hemorrhage,[1] melena, hematochezia, and resulting anemia.[1,3] The clinical

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procedure of endoscopy has shown the presence of AEC in the upper GI tract,[1] small bowel,[1,3] descending colon,[1,4] and linked their existence to upper and lower GI hemorrhage.[1,5] AECs are also significantly correlated with occurrence of synchronous lesions[6–8] and aging.[1,9] The clinical detection of AEC has increased using push-enteroscopy, capsule enterography, colonoscopy, and esophagogastroduodenoscopy, and management of these lesions is difficult with options for treatment being suboptimal.[1,10,11] Currently, endoscopic ablation is an option for lesions within endoscopic reach, whereas angiogenesis inhibitors, such as thalidomide, lenalidomide (thalidomide derivative), and octreotide, are pharmacological agents additionally used in the treatment of AEC often with limited clinical benefit.[11,12]

The precise pathophysiology of AEC is unknown; however, AECs are known to result from an imbalance between the pro-angiogenic and anti-angiogenic factors and expression of growth factors, including VEGF in AECs, is suggestive of angiogenesis playing a role in their development.[1] Angiogenesis promotes formation of new functional microvascular networks in human tissues in response to hypoxia or ischemia.[1] AEC formation appears to be linked to patients with von Willebrand factor in Heyde's syndrome and left ventricular assist device, whereas mutations in several genes in the TGFβ pathway are common in patients with hereditary hemorrhagic telangiectasia.[13] The VEGF-dependent proliferation and migration represents an important angiogenesis-hemostasis relationship that may have therapeutic implications in the management of AEC.[1,14–16] Understanding the role of key mediators in AEC development will be important in identifying novel therapeutic strategies that will overcome this unmet clinical need.

In this report, we describe a novel integrative systems biology-based approach and clinical validation study that evaluates the pathophysiology of these lesions. We sought to identify if reduction in severity or decrease in rate of AEC and AEC-correlated events occurred with use of specific drugs, hence using the medication's own mechanism of action to ascertain a "first-cut" of the inflammatory processes at work *in vivo*. To understand how therapeutic agents may impact AEC-associated disease pathology, we used *in silico* drug discovery and gene regulatory networks analysis to identify key pathways/proteins involved in the pathophysiology of AEC and test candidate therapeutics for their protective mechanisms.

METHODS

Data mining of large-scale clinical effects and hypothesis generation

US Food and Drug Administration's Adverse Event Reporting System data and University of Cincinnati Health electronic health records. Adverse events data reported to the US Food and Drug Administration (FDA)'s Adverse Event Reporting System (FAERS) and normalized within AERS*Mine*[17] was used to identify differential rates of GI hemorrhage (both upper and lower), occult blood, melena, hematochezia, and anemia, events typically associated with AEC pathology. We analyzed ~ 12 million patient reports for the FDA-approved drugs that were inversely correlated (relative risk ≤ 2, safety signal < 0) with these AEC phenotypes. Standard pharmacovigilance metrics, relative risks, and safety signals,[18,19] were used to identify drugs correlated with AEC-linked GI events. Based on "real-world" clinical experience and literature evidence, analysis was limited to adults (25–65 years) and elderly patients (> 65 years),[20,21] individuals with any history of cancer and inflammatory bowel disease (IBD) were excluded from all cohorts. To minimize confounder GI-complications, patients reporting any nonsteroidal anti-inflammatory drugs intake were also excluded.

TriNetX, a global federated health research network providing access to statistics on electronic medical records (diagnoses, procedures, medications, laboratory values, and genomic information) was used to mine ~ 1.1 million electronic health records (EHRs) from University of Cincinnati (UC) Hospital and validate FAERS findings. As a federated network, TriNetX received a waiver from Western institutional review boards, because only aggregated counts, statistical summaries of de-identified information, but no protected health information is received, and no study-specific activities are performed in retrospective analyses. The differential rates of AEC and correlated events were compared between patients on one representative PPARγ agonist (rosiglitazone, n = 180) and controls (diabetics not on PPARγ agonist, n = 68,870). Propensity score-based matching was used to balance the cohorts using age and body mass index (BMI) as covariates. Both cohorts were controlled for hypertension. AEC events included in the analysis were angiodysplasia of the colon (International Classification of Disease 10th revision codes: K55.2, K55.20, and K55.21) or arteriovenous malformation of digestive system vessel (International Classification of Disease 10th revision: Q27.33) occurring after the first instance of the respective medication in the patients' records.

Gene-regulatory biological networks. Drug-gene interactions for rosiglitazone and SGLT2 inhibitors were downloaded from STITCH,[22] PPAR signaling pathway genes were downloaded from Kyoto Encyclopedia of Genes and Genomes pathway database[23] and PPARγ interactions were downloaded from GATACA.[24] Significant AEC-linked GI phenotypes, such as GI hemorrhage, occult blood (including melena and hematochezia), were used to identify GI bleeding-associated mouse knockout models[24] and construct a biological network representation of gene functional associations and interactions to identify a set of biologically significant pathways that may be key to novel therapeutic intervention. The drug-gene interactions and phenotype-gene relationships were comparatively enriched using the ToppGene suite and ToppCluster.[25,26] See **Table S3** for gene lists used for biological network construction.

NCBI GEO. Illumina's BaseSpace Correlation Engine was used to mine NCBI's GEO and identify differential expression across *in silico*-identified genes.[27,28] The BaseSpace Correlation Engine, maintained by Illumina, San Diego, CA, collates raw experimental data from high-throughput gene expression experiments submitted to global repositories, such as the GEO[29] and Array Express.[30] The BaseSpace Correlation Engine utilizes

proprietary statistical algorithms to convert raw experimental data into a list of genes that are differentially expressed in certain conditions (drug treatment vs. nontreatment or disease state vs. normal state) along with their corresponding fold change and *P* value calculations indicating how a given gene is differentially expressed in a test condition compared with the control condition of an experiment. Rank-based enrichment statistics are then used to compute the pairwise correlation scores between all gene expression signatures present in the database. The most correlated gene expression study present for each query was assigned a numerical score of 100, and scores for the rest of the results were normalized to the top-ranked study. We limited our search to GEO datasets focusing on the human colon and identified biosets that represented differential expression across "diseased vs. normal" and "untreated vs. drug treated" colon tissues.[31–35]

In vitro study design

Patient samples and ethics approval. Patients aged 18 years and over with suspected or known GI bleeds were approached for consent before sedation and colonoscopy. Samples were collected under an approved UC institutional review board protocol. If AECs were seen, biopsies were taken from tissue immediately adjacent (referred to as involved tissue) as well as 15–20 cm away from AEC (referred to as noninvolved tissue). Biopsies from 7 control patients (no AECs) and 11 patients with AECs were collected. Four of the control patients had diabetes (further represented as DC) and three patients were nondiabetic controls (further represented as NDC). The mean age of AEC patients was 52.75 years with ages ranging from 33–74 years, whereas mean ages for DCs and NDCs were 58.75 and 58 years, respectively, (DC range 46–80 years, NDC range 50–72 years; **Table S4**). Both groups of patients had comorbidities and were on various medications at the time of endoscopy (**Tables S4–S6**). Known patients with hereditary hemorrhagic telangiectasia were excluded.

Gene expression in biopsies using relative fold differences. Mucosal biopsies were placed in RNAlater (Ambion, USA) and kept at –20°C until mRNA isolation. The mRNA was isolated using RNeasy Mini Kit (Qiagen, USA) and converted to cDNA using high capacity RNA-to-cDNA Kit (Applied Biosystems, Life Technology, USA). Analysis of gene expression was carried out with Sybr Green based quantitative real-time polymerase chain reaction. The following Quantitect Primers (Qiagen, USA) were used: Hs_VEGFA_6_SG, Hs_PPARγ_1_SG, Hs_ HIF1α_1_SG, HS_ TGFB1_1_SG, and Hs_RRN18S_1_SG. The 18sRNA was used as the internal reference gene for all samples. ΔC_T was calculated by subtracting the reference raw threshold value (18s) from the gene of interest raw threshold value (PPARγ, HIF1α, TGFβ1, or VEGFA). Relative fold calculation ($\Delta\Delta C_T$ method) was carried out using 18S as the reference gene and the NDC tissues' average as comparator for each gene. To compare all four groups against each other, 18S was the reference gene and VEGFA was the comparator.

Lectin binding and terminal sugar identification. Colon biopsies were embedded in optimum cutting temperature compound, flash frozen in a dry ice/acetone mixture, and stored at –80°C. The 7.0 μm sections were cut, and slides were fixed in 100% acetone at –20°C for 15 minutes. Slides were washed in phosphate-buffered saline (PBS), incubated in 10% fetal bovine serum/PBS for 1 hour at room temperature, and then incubated with lectin-fluorescein conjugates (Vector Laboratories, Catalog Number FLK-2100) at a final concentration of 10 μg/mL diluted in PBS-T (0.05% Tween20) for 1 hour. Slides were rinsed in PBS and then counterstained with dilute DAPI (1 μg DAPI/1 mL PBS) for 5 minutes and washed once more before air drying. ProLong Gold Anti Fade reagent (Life Technologies) was used for mounting and slides were visualized with the Zeiss LSM 710 Scanning Confocal Microscope.

The lectin-sugar binding patterns assessed were: Concanavalin A - mannose, Dolichos biflorus agglutinin - N-Acetylgalactosamine (GlcNac), Peanut Agglutinin - Galactose, Ulex Europaeus - Fucose, and Wheat Germ agglutinin - GlcNac. Binding was quantified using image J. The FITC fluorescence channel was changed to grey scale, then 15 areas per tissue were measured, 5 in the mucosal area, 5 in the epithelial barrier area, and 5 representing black background. The overall fluorescence in the mucosa and epithelial barrier was calculated as a ratio (CTCF mucosa/CTCF epithelial barrier).[36] We measured two slides for each lectin stain from three patients with AEC (both their involved and noninvolved tissues), five healthy controls, and three with IBD noninvolved "normal" areas. We used the 16-color look up table in Image J to visualize the intensity and location of the FITC-lectin binding.

Statistical methods used for patient biopsy comparisons. Box and whisker plots and *P* values were generated with the Statistix9 software. Nonparametric statistics were carried out using the Kruskal–Wallis analysis of variance and Wilcoxon rank-sum tests. The Fishers exact test was used to demonstrate differences or similarities between categorical demographic and concomitant medications.

RESULTS

Novel FDA-approved drugs as *in silico* pathophysiologic probes for AEC

To identify novel drugs as opportunities in AEC management, we used AERS*Mine*[17] to mine ~ 12 million FAERS reports from 2004Q1 to 2018Q4 for AEC and associated clinical events, such as GI hemorrhage, melena, hematochezia, occult blood, and anemia. Because FAERS is a spontaneous reporting system, which may be subjected to over reporting in certain cases, we limited our analysis to 106,212 reports submitted by "Physician," "Pharmacist," and "Health-care Professional" only. We analyzed 490 drug-event profiles across 36 therapeutic classes in these reports for drugs representing at least 2-fold change in AEC and correlated GI events as potential candidates in AEC management (**Figure 1a**). Comparative analysis of therapeutic class safety profiles revealed that the "drugs used in diabetes" class correlated with reduced rate and risk of GI bleeding events with its mean rate lower than other drug

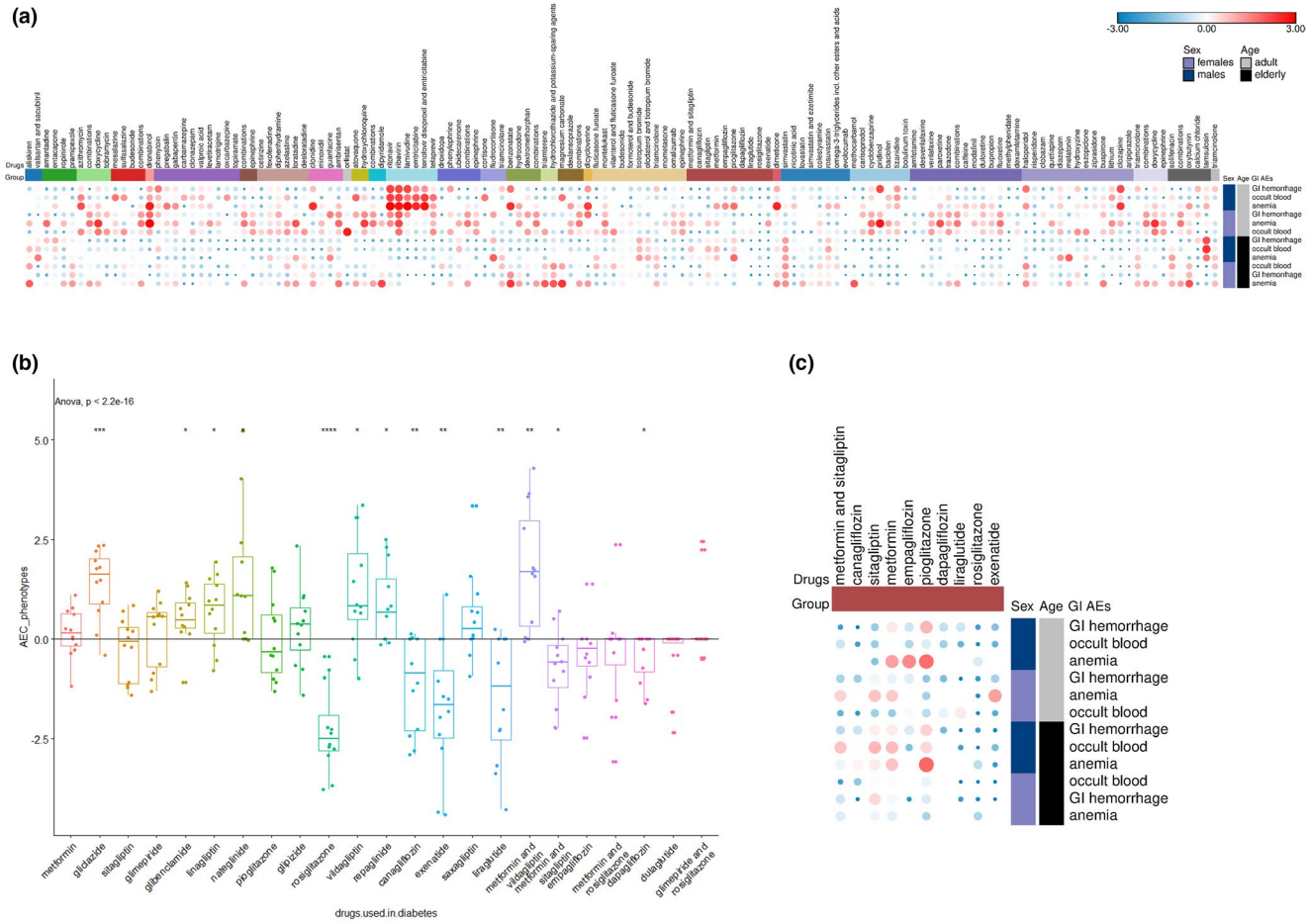


Figure 1 *In silico* discovery of potential therapeutic candidates for angioectasia (AEC). (**a**) Comparative analysis of safety signal profiles of all US Food and Drug Administration (FDA)-approved drugs across 106,212 patient reports shows differential gastrointestinal (GI)-risks across multiple therapeutic classes. The heatmap color:red = adverse risk; blue = safer toxicity profile. (**b**) Antidiabetic drugs showed favorable profiles (safety signal < 0, mean rate lower than other drug classes, $P < 0.05$) across both age (adults and elderly) and sex. Drugs with safety signal scores < 0 are classified as potential candidates. Rosiglitazone showed the safest GI profile (least rate of events) among other drugs used in the treatment of diabetes ($P < 0.0001$). (**c**) Comparative analysis of rosiglitazone, SGLT2 inhibitors and GLP-1 agonists as candidates for AEC management. Number of patients in each group: rosiglitazone (37,534), canagliflozin (10,363), exenatide (12,410), liraglutide (12,434), and empagliflozin (5,585). AE, adverse event; ANOVA, analysis of variance.

classes ($P < 0.05$; **Figure S1**, **Table S1**). Several antidiabetic drugs showed negative correlation with AEC-associated GI events, including rosiglitazone (mean safety signal: –2.3), empagliflozin (–0.4), canagliflozin (–1.1), liraglutide (–1.5), and exenatide (–1.7), with rosiglitazone showing the most significant correlation ($P < 0.0001$; **Figure 1b**, **Table S2**). In both adult and elderly patients, rosiglitazone showed the "safest" outcome profile for GI hemorrhage (both upper and lower, safety signal: –2.39), occult blood (including melena and hematochezia; –3.28), and resultant anemia (–1.2). Exenatide and liraglutide showed similar AEC profiles for GI hemorrhage (–2.3 vs. –2.6), occult blood (–2.4 vs. –1.79), and anemia (–0.3 vs. 0; **Figure 1c**, **Table S2**). Although rosiglitazone showed the most negatively correlated AEC profile, overall profiles for exenatide and liraglutide were nearly identical to rosiglitazone (**Table S2**). Recent studies have shown crosstalk between exenatide and liraglutide target GLP-1 and PPARγ, the target for rosiglitazone,[37,38] which may explain this similarity. Although the use of rosiglitazone is limited clinically,[39] we focused on it as a "straw-man" for the study of other current and future candidate drugs as pathophysiologic probes and as an option for the potential treatment of AEC.

Patient EHR data show decreased rate of AEC and associated outcomes with use of rosiglitazone

To validate FAERS analysis, we analyzed UC Hospital EHR data of 180 patients on rosiglitazone for AEC-related GI events (mean age at start of therapy 61.6 ± 12.2 years, 50% men, and 50% women). Patients with diabetes not on rosiglitazone were selected as controls (n = 68,870, mean age 57.9 ± 14.9 years, 49% men, and 51% women). Both cohorts were mutually exclusive and patients with history of cancer, IBD, Crohn's disease, or colitis were excluded from the analysis. We used one-to-one propensity score matching to balance the two cohorts. Both cohorts were controlled for age, sex, race, ethnicity, and BMI. After balancing, the mean age for the two cohorts was 61.6 ± 12.2 years, BMI

was 34.3 ± 8.68 in the rosiglitazone cohort and 35.1 ± 8.74 in the control group, and both cohorts had 180 patients. The rates of AEC and related events in the rosiglitazone cohort was 0% (no event) compared with 5.556% (n = 10) in the control cohorts (**Figure 2a**), representing a 5.556% reduction in the risk for AEC or related outcome in the rosiglitazone cohort (95% confidence interval (CI) –8.902 to –2.209, P = 0.0013; **Figure 2b**).

Representative biological network with gene functional associations and interactions

To identify potential underlying mechanisms through which AEC-associated bleeding may be mitigated, we used publicly available mouse model data to construct a biological network of drug-gene-pathway interactions. Because rosiglitazone presented the "safest" GI profile within the antidiabetic class, we sought to identify a potential PPARγ-mediated mechanism that may impact AEC-associated bleeding. AEC and associated GI events (GI hemorrhage, melena, hematochezia, occult blood, and anemia) were used to identify mouse genes whose knockout conferred phenotypes like AEC, and comparatively enriched them along with genes expressed in the PPAR signaling pathway for other mouse phenotypes, gene ontology, and pathways.[24–26] The resulting biological network representation[40] showed rosiglitazone target PPARγ significantly enriched for sets of genes vital in wound healing-associated mechanisms, TGFβ signaling pathway, regulation of epithelial cell differentiation and proliferation, regulation of vasoconstriction, angiogenesis, regulation of reactive oxygen species, and response to hypoxia and oxidative stress (**Figure 3**). Functional enrichment analysis showed that hypoxia-inducible factor 1α (HIF-1α), SERPINE1, and TNF were deeply associated with fibrinolysis, VEGF, fluid regulation, and injury response pathways implicating numerous biological processes associated with oxidative stress and wound healing in the management of AEC (**Figure 3**).

In vitro analysis of AEC biopsies

Targeted gene expression levels using nondiabetic controls as comparator. To validate the *in silico* pathway analysis, we examined gene expression levels of rosiglitazone target receptor (PPARγ), a marker of hypoxia (HIF-1α), and growth factors (TGFβ and VEGFA) in AEC and control tissue samples. We compared the relative fold expression (rfe) of these genes in four types of colonic biopsies: involved (at AEC site) and noninvolved tissue (15 cm away from AEC) from patients with AEC and non-AEC colonic tissues from DCs and NDCs.

Using NDCs as comparator, we observed that PPARγ expression in the involved AEC tissues was lower (0.55 ± 0.07) whereas the PPARγ levels in the noninvolved AEC tissues were equivalent to NDC (1.00 ± 0.11; **Figure 4a**, **Figure S2A**). DC tissues showed threefold greater PPARγ expression compared with NDCs (3.43 ± 0.77). Both AEC tissues showed statistically lower PPARγ expression compared with DCs (involved AEC P = 0.0007, noninvolved AEC P = 0.009; **Figure S2A**).

HIF1α levels varied significantly between the tissues, signifying differential oxidative stress across AEC and non-AEC samples (**Figure 4a**). Both HIF-1α and VEGFA were differentially expressed in the involved (P = 0.0019) and noninvolved (P = 0.0149) AEC tissues (**Figure 4a**, **Figure S2A**). Relative to NDC, the noninvolved AEC tissues showed higher HIF-1α levels (rfe of 1.48 ± 0.35), whereas VEGFA gene expression was lower (rfe of 0.40 ± 0.07). The levels of HIF-1α and VEGFA in the involved AEC tissue were 0.46 ± 0.04 and 0.78 ± 0.13, respectively. DC tissues showed increased HIF-1α (rfe 3.21 ± 0.90) compared with AEC tissues, particularly the involved AEC tissues (P = 0.0022; **Figure 4a**, **Figure S2A**).

VEGFA was the least expressed gene in all tissues but its expression in involved AEC (0.78 ± 0.13) was comparable to DCs (0.80 ± 0.22; **Figure 4a**). The ratio of HIF-1α/VEGFA was 56.18 ± 22.40, 54.87 ± 17.39, 8.02 ± 1.89, and 9.86 ± 0.48 for DC, noninvolved AEC, involved AEC, and NDC tissues, respectively, representing a local HIF-1α–VEGFA imbalance characteristic of AEC presentation (**Figure 4b**, **Figure S2b**). TGFβ1 expression in both involved and noninvolved AEC tissues was lower compared with NDC (rfe 0.49 ± 0.05 and 0.60 ± 0.11, respectively; **Figure 4a**, **Figure S2A**). TGFβ1 expression in DC tissue was comparable to NDC (rfe 1.31 ± 0.14). The lower expression of TGFβ1 in AEC tissues suggests that wound healing and repair may be impacted in nonhereditary AEC pathology. Collectively, the differential expression of these genes in the AEC and non-AEC human colonic biopsies present a rationale for a dysregulated relationship between oxidative stress (HIF1α and VEGFA), glycolytic/lipid/energy metabolism (PPARγ and wound healing (TGFβ1), and factors restoring the local balance will be key in the management of AEC.

Terminal glycosylation patterns as a marker of mucosal health or stress. Representative lectin/terminal sugar staining patterns showed noticeable absence of terminal epithelial fucose expression in both the involved and noninvolved AEC tissues (**Figure 5**, **Figures S3** and **S4**). UAE1/L-fucose staining was found in the vasculature of the involved and noninvolved AEC tissues. However, UEA 1 binds epithelial barrier cells in healthy intestine (**Figure 5a**, **Figure S3**) and in noninvolved IBD tissue (**Figure S4**), suggesting that terminal fucosylation in both AEC tissues is dysregulated and the luminal environment (microbiome), cell movement, and tissue healing are affected. Visually, terminal DBA/GalNAc was most intense in the epithelial cells in the healthy controls, but least intense in the involved tissue (**Figure 5A**). The WGA/GlcNAc and Concanavalin A/mannose staining patterns also differed between AEC tissue and controls (**Figures 5a,b**, **Figure S3**).

Fucose staining was increased in the mucosa of AEC tissues compared with healthy tissues (P = 0.0095; **Figure 5a,b**) and the median ratios were > 1 (**Figure 5b**). There was more GlcNAc mucosal staining in the AEC tissues as compared with controls, whereas GalNAc staining was only significantly different (P = 0.0095) in the involved tissue. There were no significant differences in mannose staining patterns.

NCBI GEO data analysis of colonic mucosa and intestinal epithelial cells

In silico identified genes PPARγ, TGFB, VEGFA, HIF-1α, glycotransferases FUTs, GALNT1 (GalNAc), and OGT (GlcNAc),

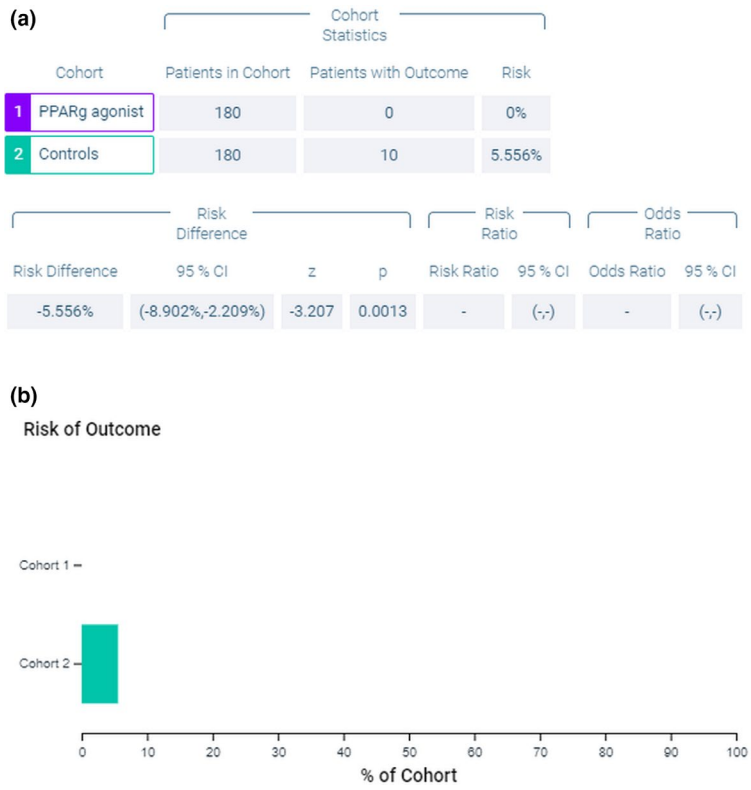


Figure 2 University of Cincinnati Hospital electronic health record (her) data analysis. (**a**) EHR data analysis shows a differential risk of angioectasia (AEC) and related events as outcome in patients on PPARγ agonist compared with controls. Patients in the PPARγ agonist cohort showed a risk difference of –5.556% for AEC (–8.902%, –2.209%, $P = 0.0013$). (**b**) A relative rate comparison of the two cohorts for their risk of AEC, 0 vs. 5.556%, for PPARγ agonist and controls, respectively. CI, confidence interval.

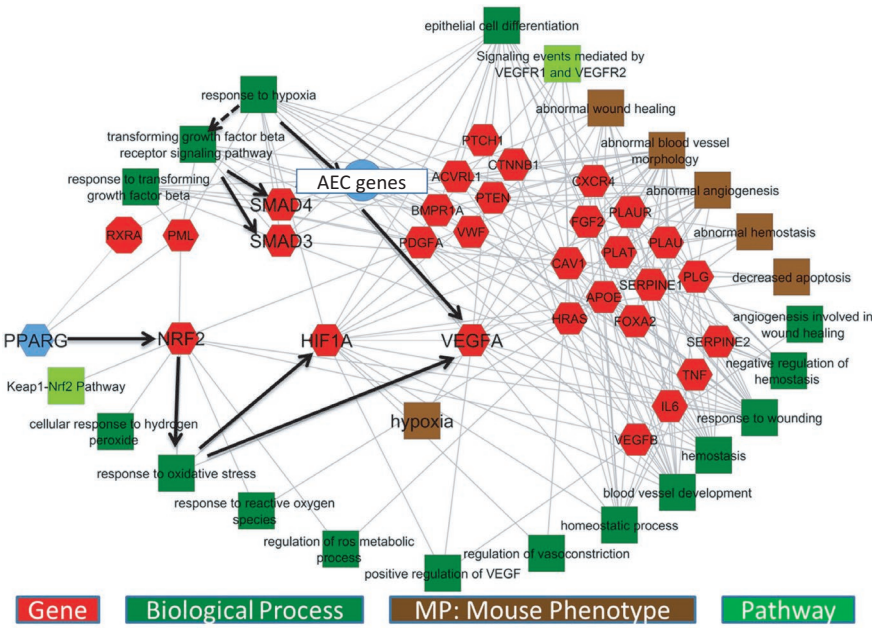


Figure 3 Biological network representation of angioectasia (AEC) pathophysiology. A high dimensional biological network modeling from 18 different mouse knockout models associated with gastrointestinal) bleeding, VEGF signaling defects, and loss of von Willebrand factor, revealed a set of linked pathways associated with increased oxidative stress and increased signaling of VEGF, FGF2, and TGF-β. These significant pathways potentially exaggerate injury responses, fluid dysregulation, and impair wound healing through abnormal blood coagulation, altered fibrin clot formation, and angiogenesis. The downstream effects of PPARγ activation on VEGF, FGF2, and TGF-β has a potent role in wound repair mechanisms.

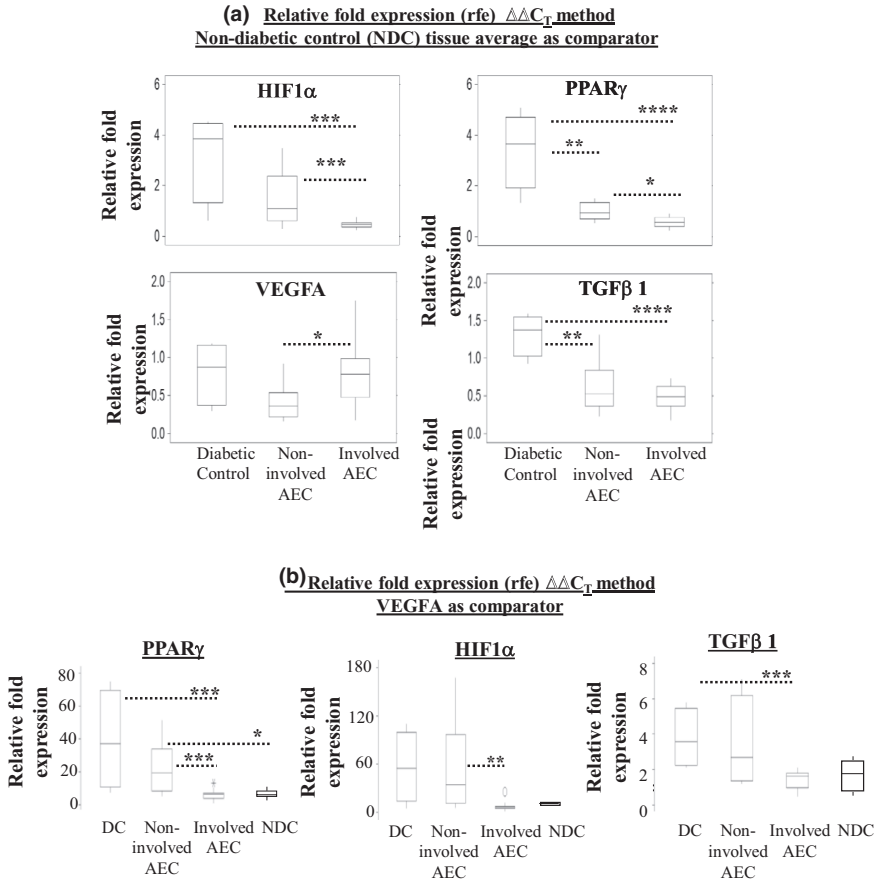
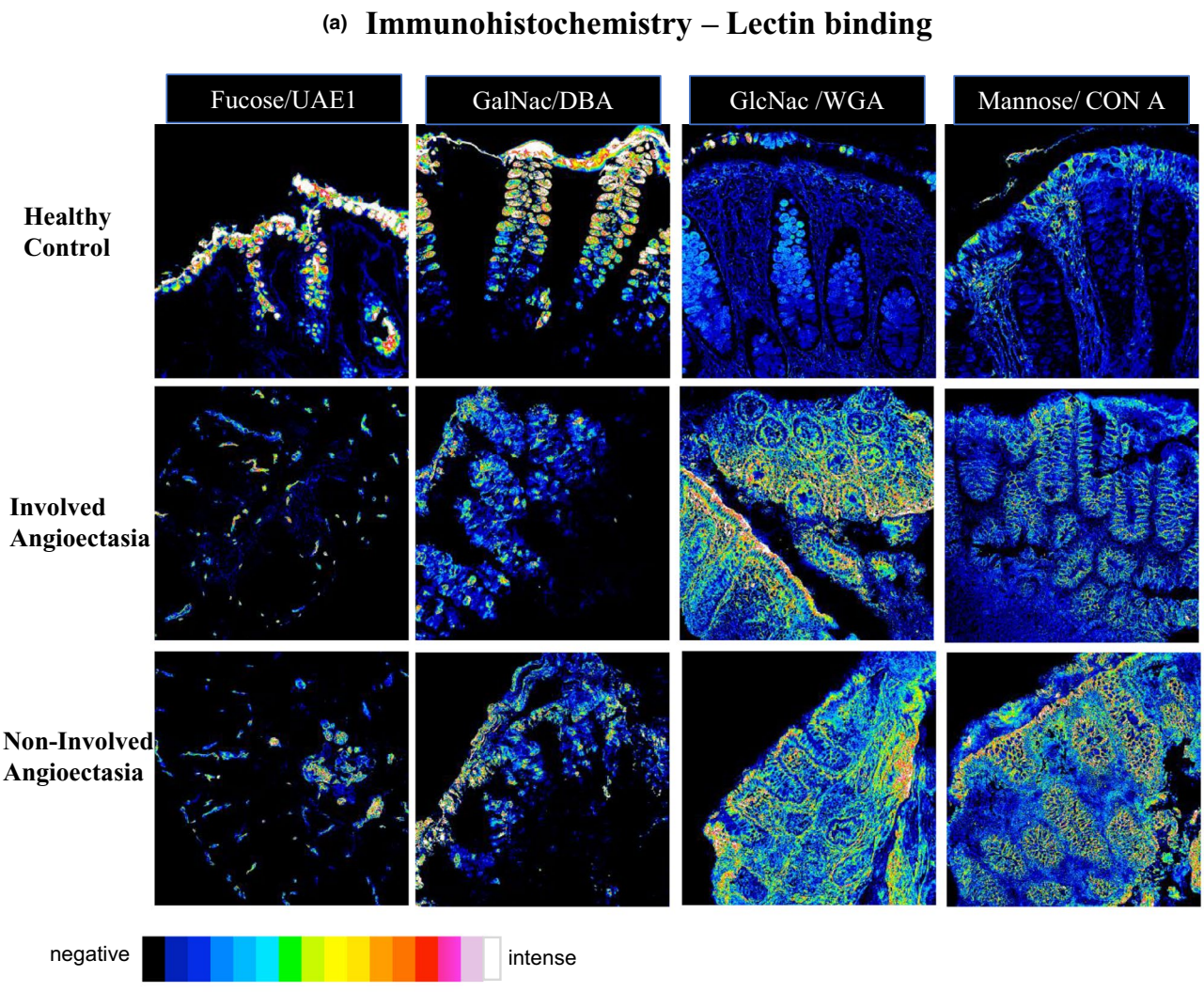


Figure 4 *In vitro* Gene Expression Using Human Colonic Biopsies. Quantification of differential gene expression using 18s rRNA gene as the internal reference. (**a**) Comparison of angioectatic tissues (AEC) and diabetic controls (DCs) to nondiabetic control (NDC) tissue using quantitative real-time polymerase chain reaction (qRT-PCR). The $\Delta\Delta C_T$ method for relative fold expression was used to compare increase or decrease of gene expression. Relative fold expression (rfe) of HIF1α, PPARγ, VEGFA, and TGFβ1 using the NDC tissue as the comparator. *$0.01 < P < 0.05$, **$0.005 < P < 0.01$, ***$0.001 < P < 0.005$, ****$0.0005 < P < 0.001$. (**b**) Comparison of all four tissue types (involved and noninvolved AEC, DC, and NDC. Relative fold expression (rfe) of HIF1α, PPARβ, and TGFβ1 using VEGFA as the comparator. VEGFA was the gene least expressed in all tissue samples. *$0.01 < P < 0.05$, **$0.005 < P < 0.01$, ***$0.001 < P < 0.005$.

and SMADs showed differential expression across disease vs. normal tissue (**Figure 6**). Increased HIF-1α correlated with decreased PPARγ and increased TGFB1 expression, in patients with ulcerative colitis or Crohn's disease compared with healthy patients (**Figure 6**, columns 7–10). PPARγ, NFE2L2 (master regulator of oxidative stress, NRF2), VEGFA, TGFB1, and SMAD4 showed correlated expression patterns in response to hypoxia. Hypoxia-correlated expression patterns were seen in FUTs, OGT, and GALNT1. The gene expression pattern most closely related to that seen in AEC tissue was the inactive UC specimen compared with healthy controls (column 11, **Figure 4**) and suggests a unique AEC characteristic of increased hypoxia, decreased PPARγ, decreased VEGFA, and glycosylation pattern.

DISCUSSION

Intestinal AECs are the most common vascular abnormality of the GI tract and the second most common cause of GI bleeding.[1] However, a lack of noninvasive treatment options in the current standards of AEC management reflects our lack of understanding of the pathophysiology of these lesions. To evaluate a potential role of the ameliorating effects of FDA-approved drugs on the clinical symptom of bleeding by AEC, we performed a large-scale mining of the FAERS and UC Hospital EHR data. Our analysis focused on the FDA-approved therapeutics for their decreased risk of intestinal bleeding events and identified antidiabetic drugs—PPARγ agonist (rosiglitazone) and glucagon-like peptide 1 receptor (GLP-1) agonists, as potential therapeutic probes in AEC management (**Figures 1b,c**, **Table S2**). Recent studies have shown that GLP-1 has the potential to induce PPARγ activity, partially explaining the anti-inflammatory effects of GLP-1 on endothelial cells.[37,38] SGLT-2 inhibitors, another class of antidiabetics, also showed decreased risk of intestinal bleeding (**Tables S1** and **S2**). Our *in silico* analysis implicated PPARγ pathway as central to AEC etiology and management, which was supported by the *in vitro* human intestinal tissue analysis. EHR analysis showed patients on PPARG agonist therapy had a 5.5% reduction in risk of AEC or associated phenotypes (**Figure 2**) paralleling our FAERS observations. Although rosiglitazone and pioglitazone both target PPARγ, we did not see a significant



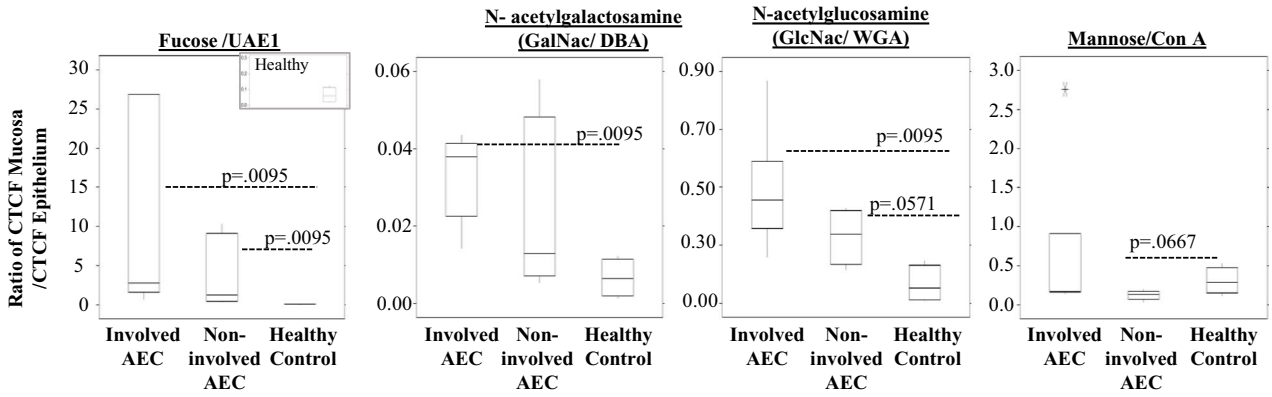


Figure 5 Lectin immunohistochemistry of angioectasia (AEC) and control tissues. (**a**) Tissues were stained with FITC labeled lectin to determine glycosylation patterns. This picture shows representative FITC-lectin binding patterns from AEC involved, AEC noninvolved, and healthy control biopsied colonic tissues. To better visualize the differential intensity of staining patterns, the images were analyzed and presented using the 16 color look up table (LUT) in image J. The scale shows most intense stain is white, while no intensity is black. (**b**) To better quantify the fluorescent intensity of the lectin staining we used the corrected total cellular fluorescence (CFTE) method and image J. The ratio of the mucosal fluorescence intensity/ epithelial barrier fluorescence intensity is presented as box and whiskers. A ratio > 1 is representative of more mucosal staining. If the ratio was < 1, then greater intensity was in the epithelial barrier. DBA, Dolichos biflorus agglutinin; WGA, Wheat Germ agglutinin.

negative correlation for AEC with pioglitazone (**Figure 1c**, **Table S2**). This may be due to pioglitazone acting like a partial PPARα agonist *in vitro*, whereas rosiglitazone seems to be a pure PPAR-gamma agonist.[41] Clearly, a targeted therapy for AEC is needed, one that integrates mechanism of action of drugs with the clinical impact of these medications on AEC bleeding.[42,43]

Our *in vitro* findings are consistent with earlier studies that have shown increased VEGF and basic fibroblast growth factor expression in human colonic AEC along with increased VEGF in circulating plasma.[44,45] In this study, we observed increased VEGFA expression in the involved (AEC) tissue, whereas expression of HIF1α was increased in noninvolved tissue (tissue surrounding the AEC). Wound healing mechanisms appear to be impaired in the AEC pathophysiology as demonstrated by decreased TGFβ1 expression both around the site of the AEC and the noninvolved AEC tissues (**Figure S2a,b**). The juxtaposition of differential angiogenic states suggests that other regional factors in the host biosystem, including Heyde's syndrome, the local microbiome, and surrounding metabolic environment, may be involved in AEC pathophysiology.[13,46–48] This complex interplay of genes, biological processes, phenotypes, and pathways is summarized through our theory of the involved gene-regulatory network (**Figure 3**).

Our preliminary work is consistent with the involvement of hypoxia and TGFβ, known to regulate VEGF-mediated angiogenesis promoting neovascularization and proliferation. Loss of HIF-1α has been found to impair this process.[49] TGFβ secretion and signaling pathways play an important role in differentiation and maturation of vasculature, wound healing, and regulation of the extracellular matrix.[50,51] Both HIF-1α and TGFβ pathways can stimulate the expression of VEGF via the activation of Smad3 and MAPKs.[44,45,49] Decreased TGFβ expression, as demonstrated by the AEC tissues, supports an environment of abnormal vascular maturation, alterations in extracellular matrix (ECM), and inflammatory processes, whereas fibrosis is caused by overexpression of TGFβ.[50,52] Both genetic and nongenetic AECs are impacted by decreased TGFβ.

AEC biopsies showed aberrant glycosylation patterns compared with normal tissue. Hypoxic conditions, as well as changes in the expression of TGFβ, can affect and regulate glycosidases functionally altering metabolism, energy, apoptosis, and wound healing.[53] The involved tissues showed increased terminal GlcNAC (OGT) in the epithelial barrier compared with the noninvolved tissue, whereas both appeared to have increased staining in the ECM. Healthy tissues showed much less GlcNAC in ECM and luminal barrier (**Figure 5a** and **Figure S3**). This AEC glycosylation pattern is distinctive from inactive IBD tissue, where there is somewhat increased terminal GlcNAC staining in the epithelial barrier, but like healthy tissue almost none in the ECM (**Figure S4**). Inactive UC patient biopsies also had increased OGT compared with healthy

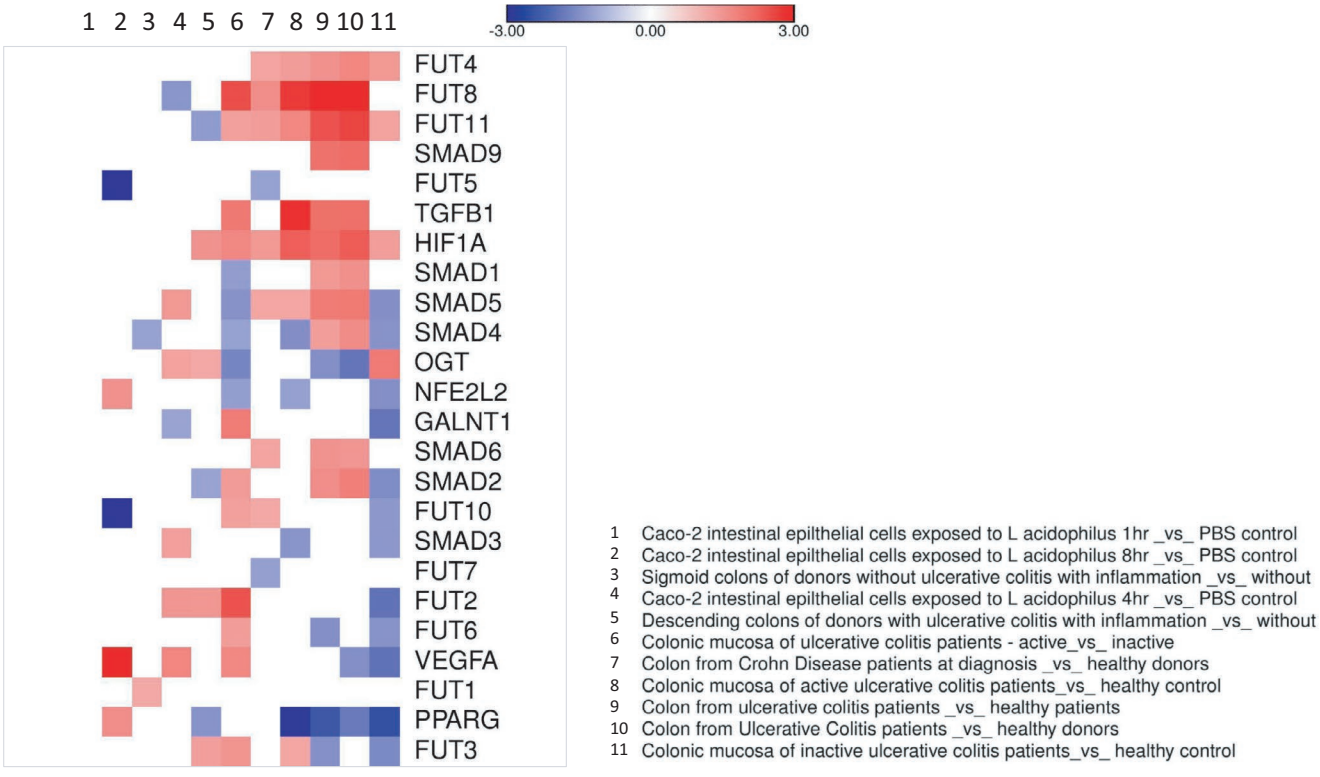


Figure 6 National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) data analysis. A large-scale comparative analysis of *in silico* identified genes PPARγ, TGFB, VEGFA, HIF-1α, glycosyltransferases (FUTs), glycosylation enzyme coding genes GALNT1 (GlcNAc) and OGT (GalNAc), including SMADs showed differential expression across disease vs. normal tissue in publicly available datasets. Datasets 6 and 11 show the differences between inactive and active ulcerative colitis, and healthy tissues. GEO Datasets: GSE9686, GSE 34175, GSE10191, GSE11223, and GSE38713.

controls and active disease (column 6, **Figure 6**). Increased O-GlcNAcylation may occur in AEC as a "short term" protective survival mechanism, becoming harmful with chronicity.[54] AEC biopsies showed increased vasculature fucose, but expression was absent on the epithelial barrier, likely due to different FUTs predominant in AEC tissues. For instance, FUT 4 and 11 were significantly increased (more than twofold) in inactive UC tissue compared with healthy, whereas all other FUTS were decreased (column 11, **Figure 6**). Active inflammation when compared with healthy or inactive inflammation shows increased FUT activity across the board (columns 6–10, **Figure 6**). AEC colon tissue did not demonstrate fibrosis or increased vascular smooth muscle cells indicative of TGFβ overexpression; decreased expression of TGFβ appeared to allow unstable vascular formation and aberrant ECM.

The lack of PPARγ expression in AEC tissues is worth noting. The involved tissues showed the lowest rfe 0.55 ± 0.07 for PPARγ, whereas both DC and noninvolved tissues had greater rfe (3.43 ± 0.77 and 1.0 ± 0.11, respectively). Changes in butyrate producing bacteria by Enterobacteriaceae have been shown to impact PPARγ signaling and may increase iNos.[55] Furthermore, PPARγ signaling shifts the metabolism of colonic epithelial cells[55] and contributions from both local host and luminal microbiome most likely impact the formation of intestinal AEC. Although we did not examine local microbiota, this will be important in future studies. Activation of PPARγ is known to induce antiproliferating effects, including cell cycle arrest and apoptosis, while regulating energy homeostasis, oxidative stress response, cell signaling, inflammation, and wound healing.[56,57] Therapies targeting PPARγ could alter energy homeostasis and in turn regulate local oxidative stress and may represent a therapeutic opportunity in AEC management. Of note, none of the current medical treatment strategies, lenalidomide or octreotide, target the PPARγ pathway.

Our study has certain limitations, some of which are inherent to our work at this stage being limited to *in silico* analyses and pilot studies based on direct hypothesis testing of control and local AEC involved colonic tissues. FAERS data has limitations, because it is a spontaneous reporting system, there is possibility for potential reporting biases and confounding, which may impact true correlations and limit hypothesis generation. Second, FAERS data lacks drug dosing information, so we are unable to analyze dose-related changes in outcomes. Patient comorbidities also have the potential to drive differential outcomes. For gene expression analysis, we only chose representative biomarkers for each pathway, PPAR signaling pathway (PPARγ), wound healing (TGFβ), vasculature (VEGF), and oxidative stress (HIF-1α), as identified through the *in silico* network analysis, is preliminary and has limitations. Clearly, a direct functional approach with *in vivo* validation is required to validate the conclusions and insights identified by this current study. Clinically, obscure intestinal bleeding management by current endoscopic and therapeutic modalities can provide some symptomatic bleeding relief, but do not address the primary AEC etiology.

We have demonstrated through an integrative analysis that combining phenotypes, genes, pathways, biological processes, and drug effects can allow for the creation of a mechanistic disease model for AEC development and growth. Our *in vitro* human tissue quantitative real-time polymerase chain reaction results and *in silico* NCBI GEO data analysis suggest that AEC pathophysiology appears to be distinct in comparison with other inflammatory GI disorders, such as Crohn's disease and ulcerative colitis.

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