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ORIGINAL RESEARCH—BASIC

Increased PXR and Suppressed T-Cell Signaling Are Associated With Malignant Degeneration of Barrett's Esophagus

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BACKGROUND AND AIMS: Barrett's esophagus (BE) is the precursor lesion for esophageal adenocarcinoma (EAC). To detect EAC in early stage, patients with BE undergo endoscopic surveillance. Surveillance cohorts largely consist of nondysplastic BE (NDBE) patients with a low annual progression risk (<0.5%). Predictive biomarkers for malignant progression of NDBE could improve efficacy of surveillance. Biomarker research has mostly focused on aberrant protein expression on BE epithelial cells. Moreover, insight in cell signaling driving malignant transformation is unknown. This study uses a data-driven approach to analyze tumor-stroma interaction in NDBE which progressed to high-grade dysplasia or EAC. **METHODS:** In this case-control study, we performed RNA sequencing analysis on index NDBE biopsies from 6 patients who, during long-term follow-up, progressed and 7 who did not progress to high-grade dysplasia/EAC. For control samples, squamous and duodenum tissues from BE patients were analyzed. For validation, we used quantitative PCR. **RESULTS:** Significant differences in BE transcriptomic profiles between progressors and nonprogressors were found by principal component and differential expression analyses. Ingenuity pathway analysis indicated that 8 cell signaling pathways were significantly upregulated in the progressors, and 14 pathways were significantly downregulated. The most interesting finding was the upregulation of the xenobiotic metabolism pregnane X receptor signaling pathway in the progressor cohort, while of the downregulated pathways in progressors, several were related to the immune system. **CONCLUSION:** These novel transcriptomic insights are fundamental for developing (chemo-)preventive therapies. These could be therapies, which protect against toxins, including biles, responsible for pregnane X receptor activation or which enhance protective immune mechanisms. The identified RNA markers are promising biomarkers for improving risk stratification in surveillance programs.

Keywords: Biomarkers; Barrett's Esophagus; RNA-seq; PXR

Introduction

Barrett's esophagus (BE) is the precursor lesion for esophageal adenocarcinoma (EAC). In BE, the normal esophageal squamous lining is replaced by abnormal intestinal-like columnar mucosa, as a result of gastroesophageal reflux disease (GERD). Patients with BE undergo endoscopic surveillance to detect and treat malignancies in early stage. The vast majority of BE surveillance cohorts consist of patients with nondysplastic BE (NDBE). Patients with NDBE have a relatively low risk to progress to high-grade dysplasia (HGD) or EAC. The annual frequency of malignant progression of NDBE is between 0.9% and 1.0% in endoscopic surveillance series, but much lower in series from national registries.[1] As a result, the cost-effectiveness of endoscopic surveillance programs for NDBE patients is debated.[2] Biomarkers that could predict malignant progression of NDBE are therefore urgently required. In the past, hypothesis-based rather than data-driven research has been conducted to identify candidate biomarkers in BE at the gene expression/protein level.[3-5] The most important biomarker reported so far by cohort and case-control studies is dysregulated expression of the tumor suppressor P53, which is due to P53 gene mutations or gene loss. Currently, overexpression of mutated P53 or complete loss of P53 gene expression due to allelic loss assessed by immunohistochemistry is the only biomarker used to risk stratify BE patients in clinical practice. The problem is that current biomarkers solely based on epithelial aberrations seem not to be able to accurately predict progression of NDBE. P53 mutations

**Shared first authorship.*

Abbreviations used in this paper: **BE, Barrett's esophagus; COX, cyclooxygenase; EAC, esophageal adenocarcinoma; GERD, gastroesophageal reflux disease; HGD, high-grade dysplasia; IQR, interquartile range; NDBE, non-dysplastic Barrett's esophagus; PPI, proton pump inhibitors.**

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occur at relatively late stage during malignant progression and mostly appear in dysplastic BE or shortly before dysplasia or cancer occurs.[6] P53 mutation and overexpression is generally not observed in NDBE patients and seems to have limited prognostic value in this subgroup of BE patients. This group of patients has a low frequency of progression in combination with long progression intervals.

Until now, most biomarker research in BE has focused on alterations at the level of the epithelial BE cells. Several studies also demonstrated that stromal factors excreted by nonepithelial cells within the BE mucosa may play a role during the malignant progression of BE.[7] Therefore, the role of the microenvironment of BE epithelium for predicting disease outcome deserves to be further investigated. Here, we hypothesized that analysis of factors expressed by both epithelial and stromal cells within the BE mucosa at the gene expression level could yield important information with respect to the malignant progression of BE. Such an analysis may lead to the identification of biomarkers and the discovery of critical signaling pathways. Although the sequential genetic mutations in the DNA of epithelial BE cells conferring a biological advantage to a subset of cells play a role in malignant transformation in BE,[8,9] insight into earliest biological mechanisms and pathways which drive this process is greatly unknown. It is very likely that crosstalk between epithelial cells and the surrounding stroma, including fibroblasts, vasculature, and immune cells, has critical roles in the onset of the malignant progression of BE. Similar crosstalk plays a critical role in the development of other cancer types.[8,10]

In a study of Owen et al, RNA sequencing profiles of bulk BE tissues and single BE cells showed the existence of distinct cell populations.[11] Interestingly, the RNA sequencing profiles which characterized BE epithelial cells proved to overlap with esophageal submucosal gland cells and were marked by expression of LEFTY1 and OLFM4.[11] These transcriptomic analyses also showed that SPINK4 and ITLN1 are markers for goblet cells, and their presence might be involved in the development of BE.[11] However, transcriptomic markers related to disease progression to cancer were not identified in this study.

In this study, our goal was to increase the insight into the pathophysiology predisposing to carcinogenesis in NDBE. We hypothesized that the microenvironment surrounding the epithelial cells is an integral part of the precancer biology, and dysregulation of specific signaling pathways within both the epithelium and the microenvironment is involved early on during the malignant degeneration of BE. The aim of this study was to elucidate which specific pathways associated with malignant progression are dysregulated in epithelial cells and the surrounding stroma in the nondysplastic stage. In this case-control study, RNA sequencing analysis of NDBE biopsies was performed to quantify large numbers of genes in both the epithelial and stromal compartments. The pathways identified in this study may offer new candidate biomarkers but also potential targets for preventive therapies in order to reduce patient risk on developing cancer.

Results

Patient Characteristics

Thirteen BE tissue biopsies from unique patients who were in surveillance programs, of whom 6 were defined as long-term progressors and 7 as long-term nonprogressors, were analyzed in the study. None of the patients had any visible signs of reflux during endoscopy at the time of biopsies. Most patients were male (83.3% and 85.7% in progressors and nonprogressors, respectively) with a mean age of 60 years (standard deviation 10.4) for progressors and 50 years (standard deviation 8.4) for nonprogressors.

The median circumferential BE segment length (5.0 cm [interquartile range {IQR} 1.0] and 3.0 cm [IQR 2.3]) was significantly different between the progressors and the nonprogressors ($P = .048$). In the progressor group, the mean time between date of the index biopsy and the date of progression was 5 years (IQR 6). A graphical view of times between index biopsies, surveillance endoscopies, and progression is shown in Figure 1. For the nonprogressors, the mean time between the date of biopsy and the last date of follow-up was 8 years (IQR 3) (Table 1). Patient characteristics are shown in Table 1. Squamous and duodenal tissues served as controls and were also sequenced.

Principal Component Analysis

After RNA sequencing data reduction of all protein-coding genes was performed by obtaining a set of principal components, a clear difference between BE tissue from progressors and that from nonprogressors was observed when samples were plotted on principal components 3 and 4 (Figure 2), but not on principal components 1 and 2 (Figure A1). This suggests that clear differences in transcriptomic profiles between the 2 groups exist. The duodenal tissues, which were basically used for control purposes from progressors and nonprogressors, showed significant overlap on PC1, PC2, PC3, and PC4, suggesting they have similar transcriptomic profiles. Results similar to those of the duodenal tissues were seen for the squamous tissues.

Differentially Expressed Genes Between Progressors and Nonprogressors

Differential expression analysis showed that 1446 genes were differentially expressed, of which 751 genes were upregulated in BE biopsies from progressors vs nonprogressors. As suggested by the principal component analyses, there were minimal transcriptional differences between duodenal squamous tissues from progressors and those from nonprogressors. Between progressors and nonprogressors, a total of only 5 differentially expressed genes were found for the duodenal biopsies, and among the squamous samples, only 81 were differentially expressed. The differentially

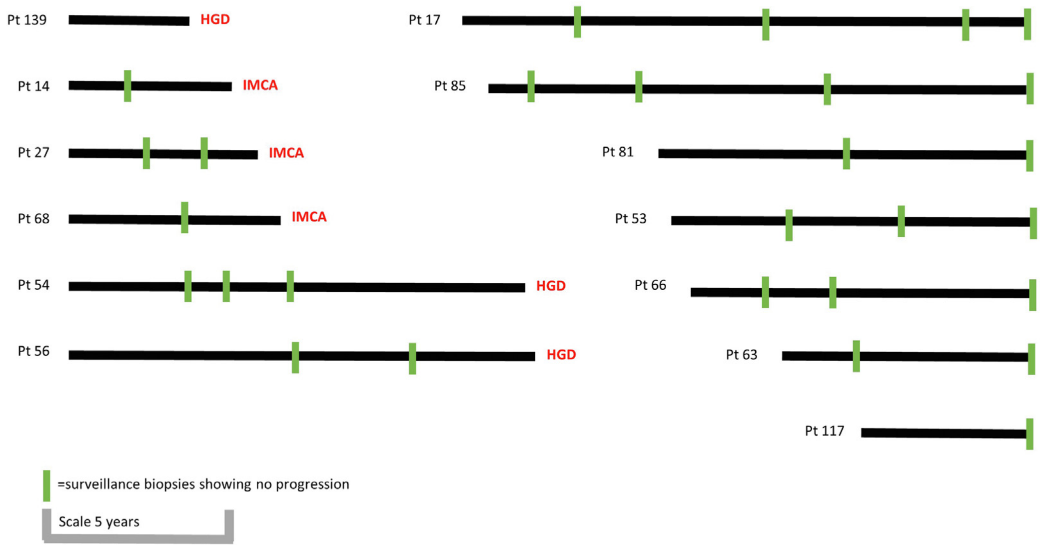

Figure 1. Time intervals between index endoscopy and surveillance endoscopies of progressors and nonprogressors. IMCA, intramucosal esophageal adenocarcinoma.

expressed genes between BE biopsies from progressors and nonprogressors are visualized in a log ratio-mean average plot (Figure 3A) and a heatmap (Figure 3B).

Dysregulated Pathways Determined by Ingenuity Pathway Analysis

Ingenuity pathway analysis (Qiagen) is a tool that enables pathway analysis on lists of differentially expressed genes resulting from comparing gene profiles between groups. Ingenuity pathway analysis (IPA) was applied to interrogate more specifically which biological processes were differentially regulated between the 2 groups (results are listed in Table A1). Eight cell signaling pathways were significantly upregulated in the progressors. These upregulated pathways in the progressors were involved in cell metabolism. These pathways included "super pathway of melatonin degradation," "nicotine degradation III," "fatty acid oxidation,"

Table 1. Patient Characteristics

Characteristics	Progressors HGD/EAC (n = 6)	Nonprogressors (n = 7)	*P*-value
Male sex fraction	83.3% ($n = 5$)	85.7% ($n = 6$)	1[a]
Mean age (±SD)	59.7 (10.4)	49.7 (8.4)	.09[b]
Median circumferential BE segment length (C) in cm (IQR)	5.0 (1.0)	3.0 (2.3)	.048[c]
Median maximum BE length (M) in cm (IQR)	4.5 (2.5)	3.0 (0.5)	.22[c]
Biopsy level 1, 2, 3 (n)	(4, 1, 1)	(7, 0, 0)	.25[d]
Mean BMI (±SD)	23.6 (±2.0)	23.3 (±1.8)	.81[b]
Use of proton pump inhibitors	100% ($n = 6$)	85.7% ($n = 6$)	1[a]
Family history of BE	16.7% ($n = 1$)	42.9% (n = 3)	.56[a]
Family history of esophageal cancer	33.3% ($n = 2$)	14.3% ($n = 1$)	.56[a]
Smoking	83.3% ($n = 5$)	57.1% ($n = 4$)	.56[a]
Median time between biopsy and progression/last time no progression years (IQR)	5 (6)	8 (3)	.8[c]

BMI, body mass index; SD, standard deviation.
[a]Fisher's exact test (2-sided).
[b]Welch 2-sample T-test (2-sided) (parametric).
[c]Wilcoxon rank sum test with continuity correction (nonparametric).
[d]Pearson's Chi-squared test.

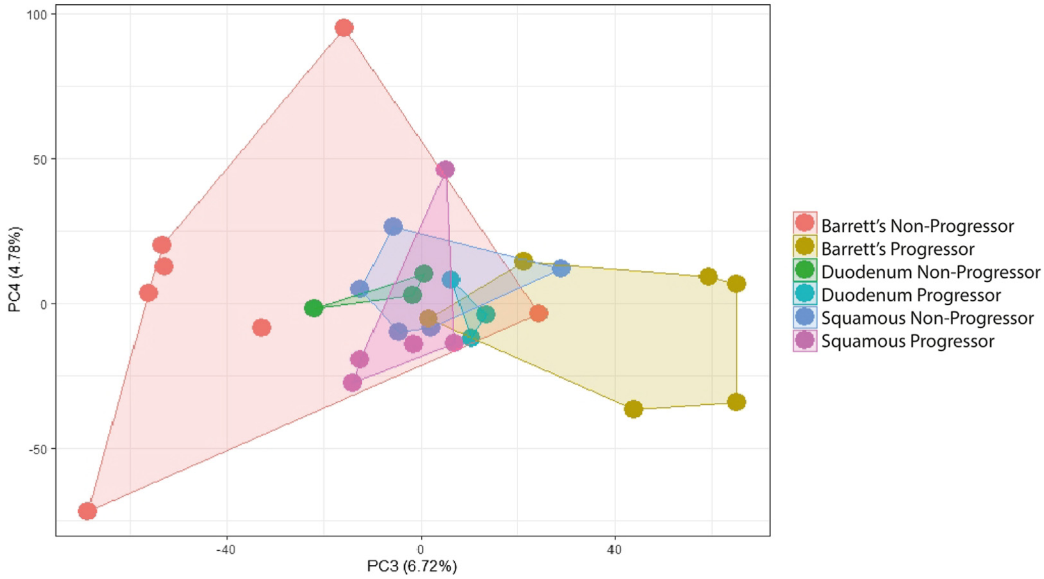

Figure 2. The principal component analysis of BE, duodenal and squamous biopsies of progressors and nonprogressors on PC3 (x-axis) and PC4 (y-axis).

"serotonin degradation," and "xenobiotix metabolism pregnane X receptor (PXR) signaling pathway" (Figure 4). PXR signaling is involved in transport of toxic agents including bile acids (Figure 5). Increased expression of PXR has been reported earlier in BE and EAC.[12]

Of interest were also those pathways that were downregulated in the progressors. Six of the 14 pathways significantly downregulated in progressors were immune pathways, including "role of nuclear factor of activated T cells in regulation of the immune response" and various signaling pathways important for T lymphocytes, interleukin (IL)-8 signaling, and "IL-15 production." Surprisingly, the pathway "regulation of the epithelial mesenchymal transition by growth factors" was lower expressed in progressors than in nonprogressors.

Validation of PXR Expression by qPCR

qPCR Analysis to validate gene expression values of PXR as found by RNA sequencing was performed for 10 BE samples (4 progressors, 6 nonprogressors) with sufficient RNA left after RNA sequencing. Gene expression quantification of PXR by RNA sequencing and qPCR on the same samples did highly correlate (Wilcoxon signed rank test $P = .004$). Gene expression values of PXR by qPCR was significantly different between progressors and nonprogressors (independent 2-group Mann-Whitney U test $P = .02$), with higher delta crossing point values and thus lower gene expression of PXR in nonprogressors (Figure 6).

Estimation of Different Types of Immune Cells Using CIBERSORT

The finding of dysregulation in inflammatory/immune signals prompted us to further interrogate the data in order to identify the different populations of cells within the BE biopsy specimens. CIBERSORT was applied to estimate the different types of immune cells. Estimated scores of abundancies showed that plasma cells ($P = .051$) and activated dendritic cells ($P = .07$) tended to be higher in nonprogressors and resting T cells, and CD4 memory cells tended to be higher in biopsies from progressors ($P = .07$).

Discussion

Surveillance cohorts of BE patients largely consist of NDBE patients who carry relatively low progression risk and generally progress after many years of follow-up. Management of this patient group requires a more advanced approach aimed at improving risk stratification and more efficient preventive and surveillance management.

In the current case-control study, we used RNA sequencing analyses and an unbiased approach to elucidate which specific pathways are upregulated or downregulated in the nondysplastic stage of BE which, after long periods of follow-up, would or would not progress to HGD or EAC. These pathways might provide insight into the background pathophysiology which early on predisposes to the malignant progression of patients with NDBE. These pathways may potentially unveil biomarkers to improve risk stratification and/or targets to improve preventive strategies.

Our most interesting finding was the upregulation of the xenobiotic metabolism PXR signaling pathway in the progressor cohort. This increased expression as observed by RNA sequencing was validated by qPCR. PXR signaling is known for its regulation of detoxification of foreign substances. Bile acids are important ligands for this nuclear receptor. The activated state of PXR signaling in potential progressors is most likely related to the fact that these NDBE patients suffer from the presence of harmful

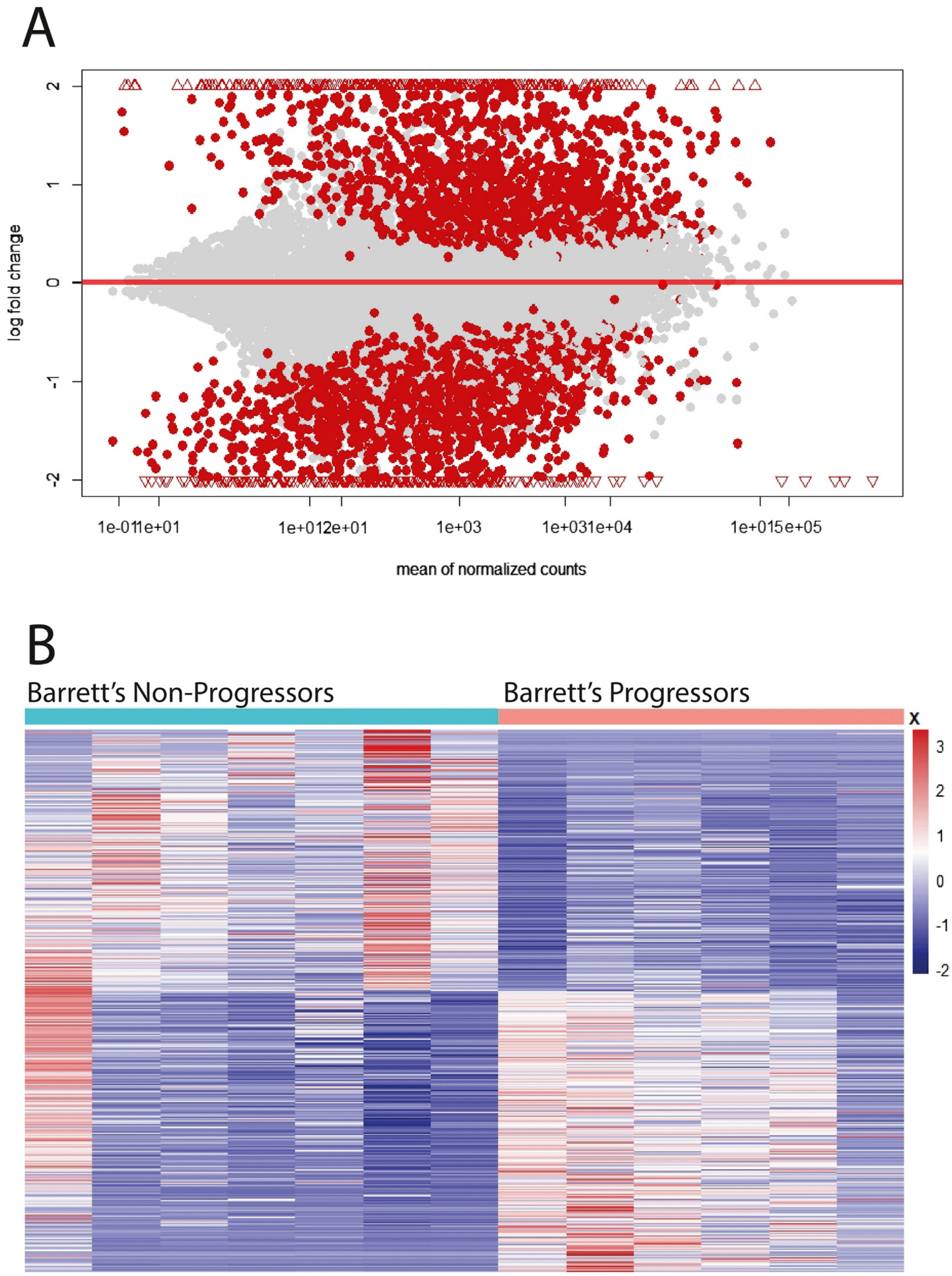

Figure 3. (A) Log ratio-mean average plots with each gene visualized as a dot (grey) and differentially expressed genes depicted by the red dots. BE tissue from progressors compared to nonprogressors has 1446 differentially expressed genes, suggesting underlying biological differences between these samples. (B) Gene expression in progressors (right side, in pink) and nonprogressors (left side, in blue). The rows depict the differentially expressed genes, and the columns depict the samples. Samples are shown in order of their tissue type (shown by the colored bar at the top). Genes are shown in order of their fold change from differential expression analysis.

chemicals as a result of GERD, leading to mucosal insults and DNA damage.[13,14]

Finding increased metabolic activity in the same progressor samples pointing to cell renewal and increased proliferation is in line with the upregulated PXR signaling. There are several factors that can explain the active PXR signaling in the progressors group. The most plausible reason is an incomplete control of bile reflux despite no signs of active reflux during endoscopy, and all patients in this study were on long-term high-dose proton pump inhibitors (PPIs). In these cases, there seems to be acid control through the use of PPI, while exposure to bile acids may

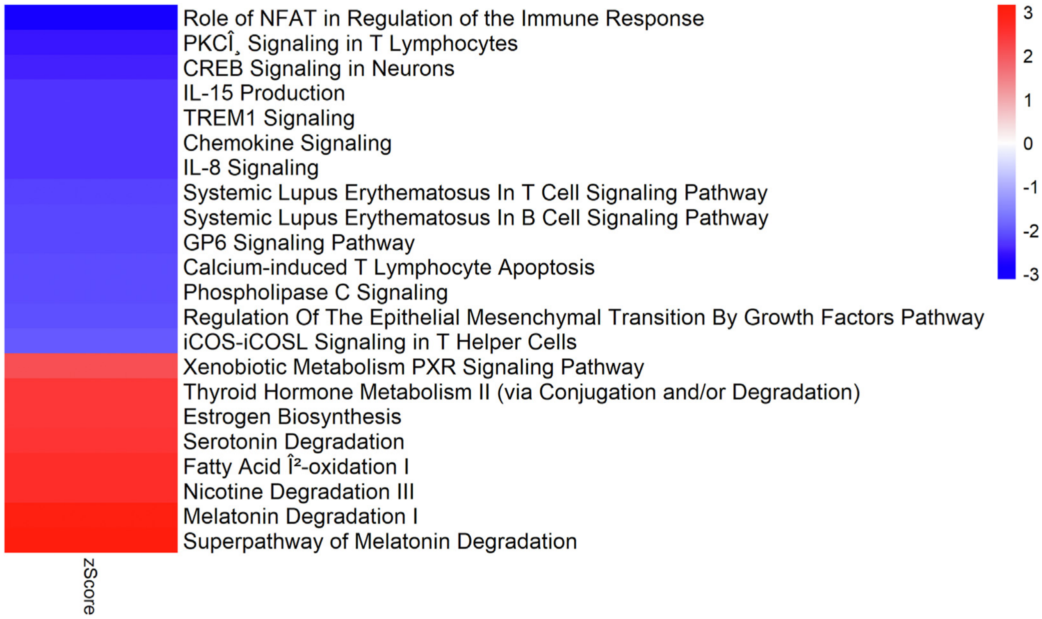

Figure 4. Ingenuity pathway analysis indicates upregulated pathways (red) and downregulated pathways (blue) in progressors vs nonprogressors. NFAT, nuclear factor of activated T cells; PKCI, protein kinase C interacting protein; CREB, cyclic adenosine monophosphate-response element binding protein; IL-15, interleukin-15; TREM1, triggering receptor expressed on myeloid cells 1; GP6, glycoprotein VI.

have persisted. Refluxates of BE patients contain more bile acids than healthy subjects, and bile acids have been demonstrated to lead to DNA damage even at neutral pH.[15-18] Moreover, our group has shown direct effects from bile acids on development of intestinal type of metaplasia which resembles human BE mucosa in a mouse model.[19] More extensive research is required to understand the exact mechanisms and bile receptors that are involved. In 1 older study, exposure of BE cells to bile acids induced translocation of PXR to the nucleus but did not cause increased PXR mRNA levels.[12] In another study on Crohn's disease by another group, it has been shown that attenuation of bile acid composition leads to differential expression of FXR and PXR.[20] If the same association exists in BE, investigation is required.

Our findings indicate that patients with active PXR signaling still have insufficient protection against chemicals and mucosal insults by bile acids. It is possible that this subgroup requires extra measures to prevent such damage. These measures could include better monitoring of bile reflux in NDBE patients and, in case of high exposure, to provide extra protective measures by combining PPI with mucosa protective agents or through changing the "aggressiveness" of the bile pool, for instance, using ursodeoxycholic acid. This would be an interesting topic of future research.

Previous research showed that patients with BE have an altered immune response compared to patients with GERD without BE. BE is characterized by an anti-inflammatory Th2-like response, rather than the proinflammatory cell-mediated cytokine profile seen in GERD.[21,22] In general, Th2-mediated immunity is associated with promotion of angiogenesis[23,24] and inhibition of cell-mediated Th1 immunity and subsequent tumor cell killing.[25] In the current study, we found that several immune pathways, including iCOS-iCOSL signaling in T helper cells and IL-15 expression, were downregulated in NDBE patients that progressed to HGD or EAC compared to patients without progression. Therefore, we conclude that a subset of patients with NDBE show disrupted immune signaling, potentially related to decreased immune surveillance.[26]

Our observation, which indicates an association between downregulation of specific immune pathways in NDBE and progression to HGD or EAC, is novel and until now received little attention. Previously, a higher risk of malignant progression has been associated with upregulated inflammatory pathways, which potentially can be suppressed by PPI and aspirin. The only clinically used pharmacological treatment to avert progression in BE is lifetime treatment with PPI, which suppresses the amount of reflux and as such decreases reflux esophagitis and potential DNA damage directly caused by acid and indirectly by bile acids.[19] Moreover, aspirin add-on to high-dose PPI improves outcomes in patients with BE[2] and protects from both EAC and esophageal squamous cell carcinoma.[27] The antitumor activity of aspirin is thought to be based on cyclooxygenase (COX)-dependent and COX-independent mechanisms. Inhibition of COX-2 and COX-2-derived prostaglandin E-2 results in inhibition of inflammation-related carcinogenesis through nuclear factor-κ B and mitogen-activated protein kinase pathways[28,29] and alteration of proliferation and apoptosis cancer pathways including mitogen-activated protein kinase, phosphoinositide 3-kinase, and cyclic adenosine

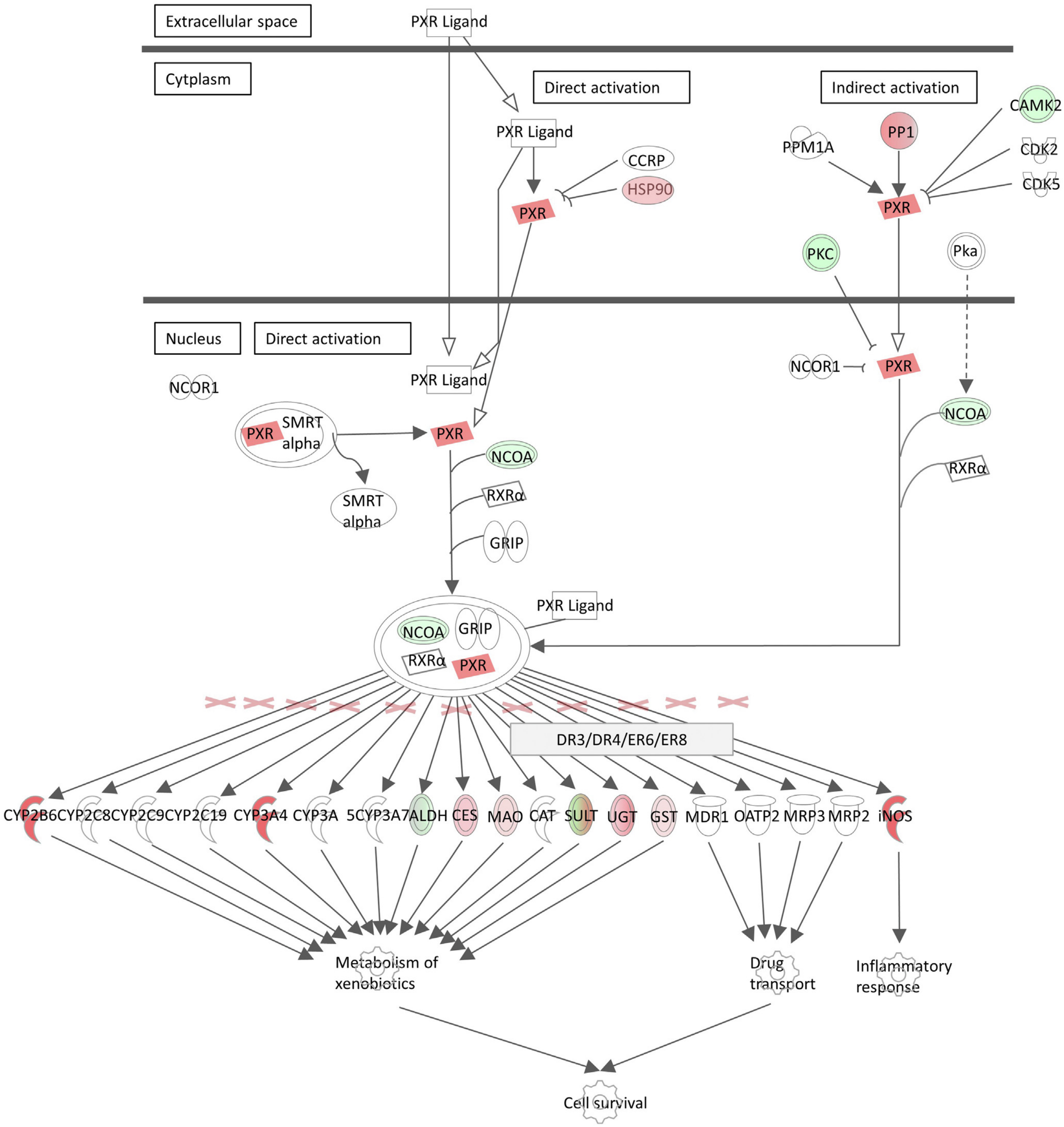

Figure 5. Graphical view of the xenobiotic metabolism PXR signaling pathway. (Adapted from: ©2000-2020 QIAGEN. All rights reserved)

monophosphate-dependent protein kinase pathways.[30] We did not see any difference between these pathways in progressors and nonprogressors. The downregulation of chemokine signaling and T-cell signaling pathways, which we identified in the progressors (who were using a high dose of PPI), indicates that these findings are independent from PPI use.

A few notes are to be made on the methodology of our paper. The number of patients analyzed by RNA sequencing is low because availability of fresh-frozen tissues required to perform high-quality RNA sequencing, from patients with NDBE before progression occurred, is limited. We decided not to use paraffin-embedded tissue for RNA expression analyses because although this type of patient material is easier to obtain, it has important limitations with regard to the quality and amount of data that can be generated.

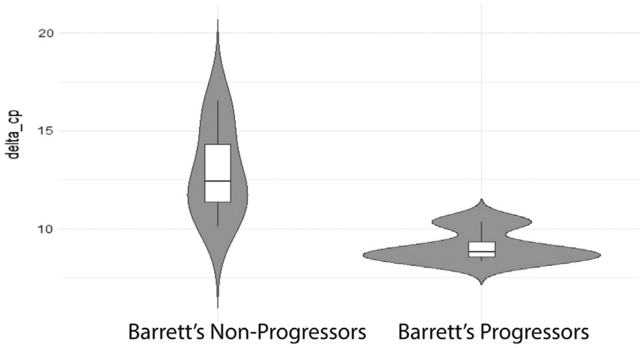

Figure 6. Violin plots showing the median (black horizontal line in white box) delta crossing point by qPCR for PXR in Barrett's nonprogressors (n = 6) (left) and Barrett's progressors (n = 4) (right), $P = .02$.

It is known that DNA methylation and its epigenetic regulatory effects on transcription alters with age.[31] However, this potential confounder could not be the case as we found no significant difference in age between progressors and nonprogressors and in RNA expression between profiles from control tissues of duodenum and squamous tissue from progressors and nonprogressors.

In summary, we showed upregulation of PXR signaling and downregulation of immune pathways to be important for T-cell regulation in BE patients that progress to EAC. These insights open the potential for preventive therapies that protect against the toxins including biles responsible for PXR activation and therapies that can boost immunosurveillance to prevent progression of NDBE to EAC. Moreover, these stroma-derived RNA markers are promising markers for further assessment of their ability to select the small group of NDBE patients that might benefit from intensified surveillance and treatment, while others may not need surveillance.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2022.08.005.

References

1. Schoofs N, Bisschops R, Prenen H. Progression of Barrett's esophagus toward esophageal adenocarcinoma: an overview. Ann Gastroenterol 2017;30(1):1–6.
2. Jankowski JAZ, de Caestecker J, Love SB, et al. Esomeprazole and aspirin in Barrett's oesophagus (AspECT): a randomised factorial trial. Lancet 2018;392(10145):400–408.
3. Martinez P, Timmer MR, Lau CT, et al. Dynamic clonal equilibrium and predetermined cancer risk in Barrett's oesophagus. Nat Commun 2016;7:12158.
4. Timmer MR, Martinez P, Lau CT, et al. Derivation of genetic biomarkers for cancer risk stratification in Barrett's oesophagus: a prospective cohort study. Gut 2016;65(10):1602–1610.
5. Stachler MD, Camarda ND, Deitrick C, et al. Detection of mutations in Barrett's esophagus before progression to high-grade dysplasia or adenocarcinoma. Gastroenterology 2018;155(1):156–167.
6. Davelaar AL, Calpe S, Lau L, et al. Aberrant TP53 detected by combining immunohistochemistry and DNA-FISH improves Barrett's esophagus progression prediction: a prospective follow-up study. Genes Chromosomes Cancer 2015;54(2):82–90.
7. Dvorak K, Dvorak B. Role of interleukin-6 in Barrett's esophagus pathogenesis. World J Gastroenterol 2013;19(15):2307–2312.
8. Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. Nat Rev Clin Oncol 2018;15(6):366–381.
9. Fitzgerald RC. Molecular basis of Barrett's oesophagus and oesophageal adenocarcinoma. Gut 2006;55(12):1810–1820.
10. Bremnes RM, Donnem T, Al-Saad S, et al. The role of tumor stroma in cancer progression and prognosis: emphasis on carcinoma-associated fibroblasts and non-small cell lung cancer. J Thorac Oncol 2011;6(1):209–217.
11. Owen RP, White MJ, Severson DT, et al. Single cell RNA-seq reveals profound transcriptional similarity between Barrett's oesophagus and oesophageal submucosal glands. Nat Commun 2018;9(1):4261.
12. van de Winkel A, Menke V, Capello A, et al. Expression, localization and polymorphisms of the nuclear receptor PXR in Barrett's esophagus and esophageal adenocarcinoma. BMC Gastroenterol 2011;11:108.
13. Wagner M, Halilbasic E, Marschall H-U, et al. CAR and PXR agonists stimulate hepatic bile acid and bilirubin detoxification and elimination pathways in mice. Hematology 2005;42:420–430.
14. Goldman A, Shahidullah M, Goldman D, et al. A novel mechanism of acid and bile acid-induced DNA damage involving Na^+/H^+ exchanger: implication for Barrett's oesophagus. Gut 2010;59(12):1606.
15. Bus P, Siersema PD, van Baal JW. Cell culture models for studying the development of Barrett's esophagus: a systematic review. Cell Oncol (Dordr) 2012;35(3):149–161.
16. Jolly AJ, Wild CP, Hardie LJ. Acid and bile salts induce DNA damage in human oesophageal cell lines. Mutagenesis 2004;19(4):319–324.
17. Kauer WK, Peters JH, DeMeester TR, et al. Mixed reflux of gastric and duodenal juices is more harmful to the esophagus than gastric juice alone. The need for surgical therapy re-emphasized. Ann Surg 1995;222(4):525–531: discussion 31-33.
18. Mason RJ, DeMeester TR. Importance of duodenogastric reflux in the surgical outpatient practice. Hepatogastroenterology 1999;46(25):48–53.
19. Straub D, Oude Elferink RPJ, Jansen PLM, et al. Glycoconjugated bile acids drive the initial metaplastic gland formation from multi-layered glands through crypt-fission in a murine model. PLoS One 2019;14(7):e0220050.
20. Wilson A, Almousa A, Teft WA, et al. Attenuation of bile acid-mediated FXR and PXR activation in patients with Crohn's disease. Sci Rep 2020;10(1):1866.

21. Souza RF, Huo X, Mittal V, et al. Gastroesophageal reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. Gastroenterology 2009;137(5):1776–1784.
22. Fitzgerald RC, Onwuegbusi BA, Bajaj-Elliott M, et al. Diversity in the oesophageal phenotypic response to gastro-oesophageal reflux: immunological determinants. Gut 2002;50(4):451–459.
23. Kodelja V, Muller C, Tenorio S, et al. Differences in angiogenic potential of classically vs alternatively activated macrophages. Immunobiology 1997; 197(5):478–493.
24. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med 1999;341(10):738–746.
25. Moons LM, Kusters JG, Bultman E, et al. Barrett's oesophagus is characterized by a predominantly humoral inflammatory response. J Pathol 2005; 207(3):269–276.
26. Ostrand-Rosenberg S. Immune surveillance: a balance between protumor and antitumor immunity. Curr Opin Genet Dev 2008;18(1):11–18.
27. Corley DA, Kerlikowske K, Verma R, et al. Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. Gastroenterology 2003;124(1):47–56.
28. Hanif R, Pittas A, Feng Y, et al. Effects of nonsteroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway. Biochem Pharmacol 1996; 52(2):237–245.
29. Yousif NG, Al-Amran FG, Hadi N, et al. Expression of IL-32 modulates NF-kappaB and p38 MAP kinase pathways in human esophageal cancer. Cytokine 2013; 61(1):223–227.
30. Lin HR, Wu YH, Yen WC, et al. Diminished COX-2/PGE2-mediated antiviral response due to impaired NOX/MAPK signaling in G6PD-knockdown lung epithelial cells. PLoS One 2016;11(4):e0153462.
31. Curtius K, Wong CJ, Hazelton WD, et al. A molecular clock infers heterogeneous tissue age among patients with Barrett's esophagus. Plos Comput Biol 2016; 12(5):e1004919.

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Authors' Contributions:
K. K. Krishnadath designed the study. S. J. M. Hoefnagel, E. M. Timmer, and S. Li performed wet lab experiments. S. J. M. Hoefnagel collected the clinical data. S. J. M. Hoefnagel and E. M. Timmer performed data management. S. J. M. Hoefnagel and K. K. Krishnadath performed the statistical data analysis and interpretation of statistical data; wrote the paper; and created the figures. All authors reviewed the drafts of the paper and gave final approval of the version to be published.

Conflicts of Interest:
The authors disclose no conflicts.

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Ethical Statement:
The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
RNA sequencing profiles will be made accessible at GEO data repository GSE207527.