RESEARCH LETTER

Barrett's Esophagus Epithelial Stem Cells Have Distinct Gene Signatures

Esophageal adenocarcinoma (EAC) has a devasting 5-year survival rate of <20%[1] and rapidly increasing incidence rates.[1] Barrett's esophagus (BE), a metaplastic condition that originates in the distal esophagus, is characterized by the replacement of squamous epithelium with columnar epithelium with gastric and intestinal features and is the only known precursor lesion for the development of EAC.[2] Although metaplasia in BE advances to low-grade dysplasia (LGD) or high-grade dysplasia (HGD) in only a subset of patients, such progression significantly increases the risk for EAC[3] and underscores the importance of identifying biomarkers of disease progression that would inform surveillance and potentially enable earlier diagnosis and treatment.

To determine potential biomarkers of BE disease progression, we analyzed epithelial stem cell organoids generated from nondysplastic BE (ND BE), LGD BE, HGD BE, BE with both HGD and EAC components (HGD BE plus EAC), and EAC (Figure A, Supplemental Methods). Principal component analysis of epithelial stem cell genes showed that ND BE and LGD BE overlapped transcriptionally and that HGD BE was transcriptionally intermediate between the ND and LGD BE cluster and EAC (Figure A). The transcriptome profile of EAC stem cells closely clustered with the profile of gastric adenocarcinoma (GAC) stem cells (Figure A), suggesting commonality, as previously reported.[4]

To elucidate the transcriptional evolution of epithelial stem cells through the stages of BE progression (Figure A), we profiled organoids generated from ND BE, LGD BE, and HGD BE for genes with biomarker potential, including cancer-associated, stemness, and immune response genes. We compared each BE group, in addition to EAC and GAC organoids, with normal cardia-derived organoids (Figure B), the likely source of metaplastic BE cells.[4,5] *CDX2*, *HOXA13*, *HOXB3*, *HOXC10*, and *PTGS2* were strongly upregulated in both ND and dysplastic BE but not in EAC (Figure B), consistent with a classical BE signature.[6] Strikingly, cancer testis antigen 83 gene (*CT83*), not previously linked to BE, showed notable specificity for BE epithelial stem cells compared with EAC and GAC (Figure B).

We next examined the stem cell panel for genes with increased expression in BE (ND and dysplastic) and EAC or only BE (ND and dysplastic) compared with normal cardia stem cells that might reflect high- or low-risk, respectively, progression to adenocarcinoma. *UCA1*, *FZD10*, *ST6GAL1*, *HOXC10*, and *SOX14* were highly expressed in BE and EAC (Figure B highlighted in burgundy), suggesting potential high-risk progression. We detected no significant difference in the expression of high-risk genes in BE- and EAC-derived organoids in an additional cohort (Figure C). Public data from The Cancer Genome Atlas (TCGA), which we analyzed using The University of Alabama at Birmingham Cancer data analysis portal,[7] confirmed elevated expression of *UCA1*, *ST6GAL1*, *HOXC10* and *SOX14*, although not *FZD10*, in both EAC and GAC (Table A1), corroborating our findings.

The genes *CT83*, *PTGS2*, *MUC7*, and *IL23A* were highly expressed in organoids generated from BE (ND and dysplastic) but markedly downregulated or expressed at a low level in EAC, suggesting potential biomarkers of BE with a low risk for progression (Figure B, highlighted in blue). *CT83*, *PTGS2*, and *IL23A* were significantly downregulated in an additional cohort of EAC-derived organoids compared with BE-derived organoids (Figure C). *MUC7* displayed a downward trend in expression but did not reach statistical significance (Figure C). The TCGA database does not report expression of *MUC7* in EAC but shows slight upregulation of *PTGS2* ($P = .047$) and significant upregulation of *IL23A* in EAC (Table A1). *CT83* is not part of the TCGA database, but has recently been reported to be overexpressed in a number of cancers and possesses notable specificity for triple negative breast cancer.[8] The high specificity of *CT83* in BE-derived organoids suggests a novel epithelial biomarker of BE. The contrasting gene expression between our results and the TCGA database likely reflects analysis of epithelial stem cells and comparison with normal gastric cardia columnar epithelial stem cells (our data) vs analysis of tissue and comparison with normal squamous esophageal tissue (TCGA).

We previously showed that expression of ST6Gal1, a sialyltransferase that plays a role in the regulation of homeostatic apoptosis, increased during gastric intestinal metaplasia and gastric cancer.[9] Due to the transcriptional similarities between BE metaplasia and gastric intestinal metaplasia,[4,5] we examined *ST6GAL1* expression as a candidate gene indicator of BE progression to EAC. *ST6GAL1* mRNA was significantly upregulated in dysplastic BE-derived organoids compared with normal cardia-derived organoids in an additional cohort (Figure D). Unexpectedly, *ST6GAL1* mRNA expression was not increased in EAC-derived organoids (Figure D). Recently, the disulfide catalyst quiescin sulfhydryl oxidase-1 (QSOX1) was shown to post-transcriptionally regulate ST6Gal1 activity depending on local tissue conditions.[10] We detected similar *QSOX1* mRNA (Figure E) and protein (Figure F) expression levels in BE- and EAC-derived organoids and tissue, respectively. The equivalent levels of QSOX1 in BE and EAC reflect a potential role for QSOX1 and suggest it may contribute to the regulation of

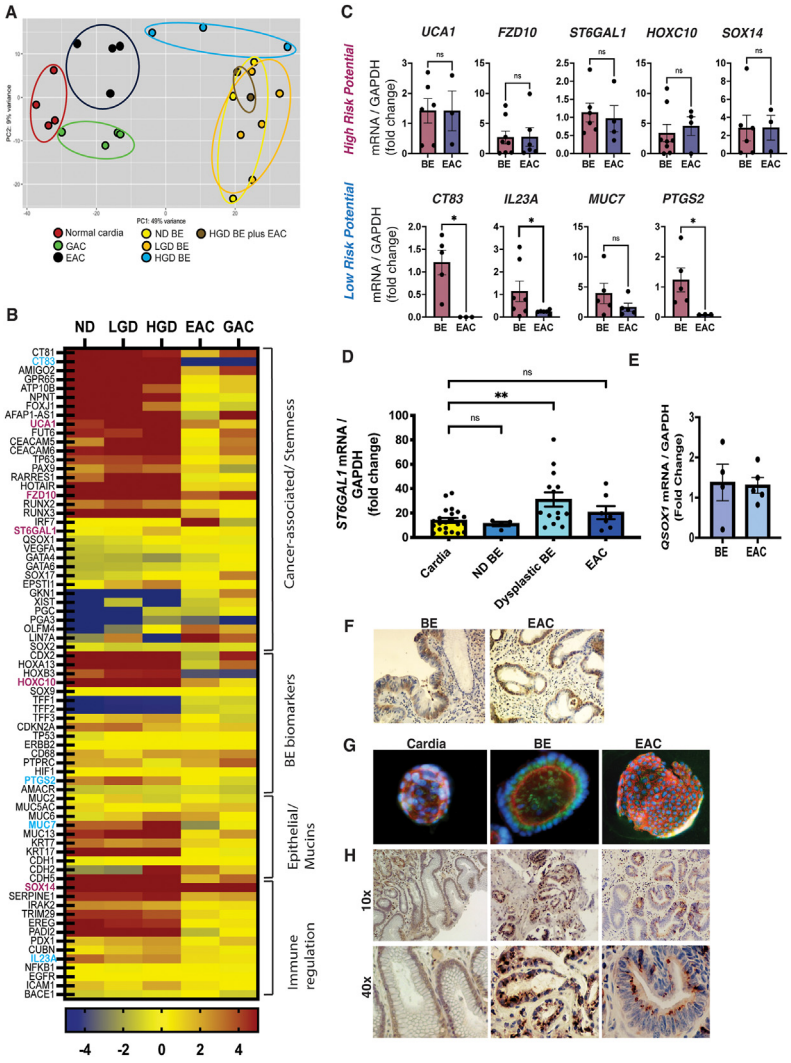

Figure. Potential high- and low-risk gene profiles for disease progression in BE. (A) β-diversity of mRNA gene expression in organoids generated from ND BE (n = 3, yellow), LGD BE (n = 5, orange), HGD BE (n = 3, blue), HGD BE and EAC (n = 2, brown), EAC (n = 4, black), GAC (n = 4, green), and normal gastric cardia (n = 4, red) determined by RNA-Seq and displayed as a PCA plot with each dot representing a single subject. (B) Heat map for epithelial stem cell gene expression in organoids generated from ND BE (n = 3), LGD BE (n = 5), HGD BE (n = 3), EAC (n = 4), and GAC (n = 4) normalized to epithelial stem cell gene expression in organoids generated from normal gastric cardia (n = 4, control). (Scale: log base 2-fold change, range −5 to 5). Potential High-risk genes are highlighted in burgundy, and potential low-risk genes are highlighted in blue. (C) Epithelial organoids generated from BE (n = 3–5) or EAC (n = 3–5) tissue were analyzed for *UCA1*, *FZD10*, *ST6GAL1*, *HOXC10*, and *SOX14* (high-risk) or *CT83*, *IL23A*, *MUC7*, and *PTGS2* (low-risk) gene expression by real time PCR (Data shown as mean ± SEM, unpaired *t* test, significance: $^{*}P < .05$). (D) Epithelial organoids generated from tissue from normal gastric cardia (n = 22, control), ND BE (n = 4), and dysplastic BE (n = 14) or EAC (n = 7) were analyzed for *ST6GAL1* gene expression by real-time PCR. (Data are shown as mean ± SEM, 1-way ANOVA, significance: $^{**}P < .005$) (E) Epithelial organoids were generated from tissue from BE (n = 4) or EAC (n = 5) and analyzed for *QSOX1* gene expression by real-time PCR. (Data shown as mean ± SEM). (F) Esophageal tissue from subjects with BE (LGD shown) or EAC was stained for QSOX1 (DAB) by immunohistochemistry (n = 3 each, representative images shown, 10×). (G) Cardia, BE- (LGD shown), or EAC-derived organoids stained with antibodies for ST6GAL1 (FITC), phalloidin (Alexa Fluor 594), and DAPI (n = 3 each, representative donors shown, 20×). (H) Cardia tissue from healthy donors or esophageal tissue from subjects with BE (LGD shown) or EAC stained with antibodies to ST6GAL1 (DAB) by immunohistochemistry (n = 3 each, representative images shown, 10× and 40×). ANOVA, analysis of variance; BE, Barrett's esophagus; DAB, 3,3'-diaminobenzidine; DAPI, 4',6-diamidino-2-phenylindole; EAC, esophageal adenocarcinoma; FITC, fluorescein isothiocyanate; GAC, gastric adenocarcinoma; HGD, high-grade dysplasia; LGD, low-grade dysplasia; mRNA, messenger RNA; ND, nondysplastic; PCA, principal component analysis; PCR, polymerase chain reaction; SEM, standard error of the mean.

ST6GAL1 activity. Organoids generated from BE expressed high levels of ST6Gal1 protein (Figure G). In contrast to the low mRNA expression of *ST6GAL1* in EAC, ST6Gal1 protein was elevated in EAC-derived organoids (Figure G), consistent with QSOX1 expression in both BE and EAC. Further, ST6Gal1 protein was present in tissue biopsies from subjects with BE and EAC (Figure H).

Currently, classification of BE is based largely on histology, which varies depending on pathologist-specific interpretation. Consequently, a biomarker or combination of biomarkers that detect subjects with BE at high risk for tumor progression would inform surveillance and early diagnosis and potentially enhance successful intervention. Here, we show *UCA1*, *FZD10*, *ST6GAL1*, *HOXC10*, and *SOX14* are highly elevated in epithelial stem cells from both BE and EAC, suggesting this panel may reflect potential for high-risk tumor progression. Further, we show *CT83*, *PTGS2*, *MUC7*, and *IL23A* are highly expressed in BE but downregulated in EAC epithelial stem cells, suggesting this panel may reflect BE with a low risk of tumor progression. Although this is a cross-sectional analysis, our findings enlarge the framework for future study of the gene biomarkers for progressing and nonprogressing BE and gene-encoded pathways involved in driving BE progression to EAC.

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Supplementary Materials

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Abbreviations used in this paper: **BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; GAC, gastric adenocarcinoma; HGD, high-grade dysplasia; LGD, low-grade dysplasia; ND BE, nondysplastic Barrett's esophagus**

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All human specimens were obtained after written informed consent approved by the University of Alabama at Birmingham Institutional review Board (UAB IRB#170317005) and abide by the Declaration of Helsinki Principles.

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