Activation of GATA binding protein 6 (GATA6) sustains oncogenic lineage-survival in esophageal adenocarcinoma

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.
Activation of GATA binding protein 6 (GATA6) sustains oncogenic lineage-survival in esophageal adenocarcinoma


*Department of Surgery, †Department of Pathology, and ‡Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109; ¶Department of Medical Oncology, Dana-Farber Cancer Institute, and Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA 02115; and ‡Department of Integrative Oncology, British Columbia Cancer Research Centre, Vancouver, BC, Canada V5Z 1L3

Edited by Joe W. Gray, Oregon Health and Science University, Portland, Oregon, and accepted by the Editorial Board January 10, 2012 (received for review August 13, 2010)

Gene amplification is a tumor-specific event during malignant transformation. Recent studies have proposed a lineage-dependency (addiction) model of human cancer whereby amplification of certain lineage transcription factors predisposes a survival mechanism in tumor cells. These tumor cells are derived from tissues where the lineage factors play essential developmental and maintenance roles. Here, we show that recurrent amplification at 18q11.2 occurs in 21% of esophageal adenocarcinomas. Utilization of an integrative genomic strategy reveals a single gene, the embryonic endoderm transcription factor GATA6, as the selected target of the amplification. Overexpression of GATA6 is found in EACs that contain gene amplification. We find that EAC patients whose tumors carry GATA6 amplification have a poorer survival. We show that ectopic expression of GATA6, together with FGFR2 isoform IIb, increases anchorage-independent growth in immortalized Barrett’s esophageal cells. Conversely, siRNA-mediated silencing of GATA6 significantly reduces both cell proliferation and anchorage-independent growth in EAC cells. We further demonstrate that induction of apoptotic/anoikis pathways is triggered upon silencing of GATA6 in EAC cells but not in esophageal squamous cells. We show that activation of p38α signaling and up-regulation of TNF-related apoptosis-inducing ligand are detected in apoptotic EAC cells upon GATA6 deprivation. We conclude that selective gene amplification of GATA6 during EAC development sustains oncogenic lineage-survival of esophageal adenocarcinoma.

Eosophageal adenocarcinoma (EAC) is a highly lethal malignancy of the distal esophagus with a 5-y survival rate of only 10–15%. The incidence of EAC has increased 300–500% in the past three decades in Western countries (1). Chronic gastroesophageal reflux disease is a major risk factor for development of Barrett’s esophagus, a condition whereby normal squamous epithelium of the distal esophagus is replaced by epithelia of intestinal metaplasia. Barrett’s esophagus may predispose patients to the development of EAC; rates of transformation to cancer are estimated at 0.5% per year for patients with Barrett’s metaplasia and 10% per year for those with dysplasia (2).

Chromosomal aneuploidy and mutations/deletions of the tumor-suppressor genes, p16/CDKN2A and TP53, are prevalent and occur early in the progression from Barrett’s metaplasia to EAC (3, 4). These somatic changes, however, are hallmarks for many, if not all, cancer types and lack specificity for EAC origin (5). DNA copy number increase is another common event in EAC, and individual amplified loci identified in EAC demonstrate tumor-type specificities that may be essential for the malignant transformation in this disease (6–8). To date, the key molecular pathways and mechanisms that underlie malignant transformation from Barrett’s metaplasia to EAC remain undetermined.

Cancer development is a multistage process involving both activation of oncogenes and inactivation of tumor suppressor genes (9). Gene amplification is one mechanism for the activation of oncogenes, and this activation can be causative for tumorigenesis (10, 11). Despite the complexity of genetic, epigenetic, and chromosomal abnormalities in a given cancer, inactivation of a single or a few initiating oncogenes may impair tumor growth and survival, a phenotype termed “oncogene addiction” (12, 13). Recent studies of genomic amplification in cancer demonstrated that certain master regulatory factors, involved in both embryogenesis and subsequent tissue maintenance, are often selectively amplified in tumors arising from the lineages where the factors play an important developmental role (14–17). In the present study, we identify a highly amplified transcription factor, GATA6, in EAC using integrative genomic approaches and show that GATA6 has properties of a lineage-survival oncogene in EAC.

Results

Integrative Genomic Analysis in EAC Identifies Recurrent Amplification at 18q11.2 and a Single Selected Target Gene, GATA6. We performed array-based comparative genomic hybridization (array-CGH) in 20 EAC samples and identified genomic amplification at 18q11.2 as a recurrently amplified locus (Fig. 1L). Three of 20 EAC samples assayed by array-CGH showed amplification with one tumor (T8) containing an amplified unit about 706 kb that included only two genes, GATA6 and CTAGE1 (Fig. 1L, and SI Appendix, Fig. S1A and Table S1A). We next analyzed DNA dosage of five genes spanning a 1.5-Mb segment of the 18q11.2 region, including both GATA6 and CTAGE1, in a cohort of 85 EACs using real-time PCR (qPCR) (Fig. 1B and SI Appendix, Fig. S2). The 2−ΔΔCt calculation of cycle threshold was performed (18). The cutoff value was arbitrarily set at ≥1.9, which represented greater than 4N of the haploid genome, given ≥70% tumor cell content of the EAC samples studied. GATA6 amplification was found in 18 of 85 EACs (21.2%), which is significantly higher than the other four genes amplified in the amplicon (P < 0.001) (Fig. 1C, and SI Appendix, Fig. S1C and Table S1C). The highest DNA copy number (>36N) was also found in the GATA6 gene.
GATA6 expression in tumors containing ≥Lin et al. (Fig. 1 <0.0001) (D) indicating the fold-change of a 2N genome and the axis lists the tumor ID, of which sample 1 is a mean normal value. Numbers in parentheses represent amplification percentages of the genes examined in 85 EACs. Yellow line highlights the cutoff value. All qPCR reactions were repeated in triplicate. (C) Boxplot of the qPCR data. GATA6 demonstrates higher interquartile range, a larger upper whisker and more extreme upper-outliers than other genes within the amplicon and shows significant difference from the other four genes (**p < 0.01, ***p < 0.001; two-tailed, paired t test). (D) Recurrent amplifications of chromosome 18q11.2 in 73 EACs from SNP array data visualized in hg18 genome build using the IGV software. The y axis shows a descending log2 copy number ratio in 73 EACs. Horizontal bars represent individual tumor samples. The boxed area shows a 4-Mb region in the vicinity of the 18q11.2 amplicon with the arrow indicating the location of GATA6. (E) Magnified view of the 4-Mb region of the 18q11.2 amplicon from D. Boxed region with yellow lines shows the smallest amplified unit defined in 73 EACs. (F) Kaplan–Meier survival plots estimate a poorer clinical outcome (P = 0.0292) in EAC patients bearing the 18q11.2 amplicon in their tumors.

Gene Amplification Drives the Overexpression of GATA6 in EACs. Transcriptional expression of GATA6 among 50 EACs, including all amplified tumor specimens available, was assayed using quantitative RT-PCR (qRT-PCR) (Fig. 2A and SI Appendix, Fig. S2C). The value of 2−ΔΔCt ≥2 (twofold) relative to normal intestinal RNA was set as the threshold for gene overexpression. Fourteen of these 30 EAC samples contained GATA6 amplification; among them, 13 (93%) were found to overexpress GATA6 (r = 0.850, P < 0.0001). Only 9 of 30 EACs were found to have MIB1 amplification (SI Appendix, Fig. S1C) and five of these nine samples overexpressed MIB1 (r = 0.873, P = 0.8406) (Fig. 2A and B). The change in GATA6 expression in tumors containing GATA6 amplification was significantly greater than that in tumors without GATA6 amplification (P < 0.001) or in tumors with or without MIB1 amplification (P < 0.001) (Fig. 2B). Overexpression of GATA6 protein in these cases was confirmed using both Western blot and immunohistochemistry with an esophageal tissue microarray (TMA) (Fig. 2C and D). Ten of 13 GATA6-amplified EAC TMA cores demonstrated strong staining, whereas only seven were found to contain MIB1 amplification and four of the seven MIB1-amplified EACs had positive MIB1 staining (Fig. 2D). Furthermore, when we analyzed a multicancer study of gene-expression profiling using the Oncomine database (www.oncmine.com), we found that GATA6 was one of the signature genes with high expression that distinguishes gastrointestinal carcinomas from other tumor types (SI Appendix, Fig. S3).

Ectopic Expression of GATA6 Increases Anchorage-Independent Growth in Immortalized Barrett’s Cells in Collaboration with FGFR2IIb. Given that GATA6 is an embryonic gut lineage transcription factor and that gene amplification of GATA6 is selected during development of EAC, we hypothesized that GATA6 exerts an oncogenic lineage-survival role in EAC. We found that GATA6 alone was not transforming, as determined by anchorage-independent assays in 3T3, RK3E, and immortalized Barrett’s CP-A (Fig. 3A) cells. We then examined whether GATA6 was transforming in collaboration with other genetic events. Analysis of our EAC U133A array data showed that expression of FGFR2, a receptor tyrosine kinase and an oncogene amplified in gastric cancer (19), was one of the top 50 genes significantly correlated with GATA6 expression (r = 0.58, P < 0.0001) (SI Appendix, Fig. S4A). We also found that FGFR2 and GATA6 were coamplified in one EAC (SI Appendix, Fig. S4B). Additionally, we recently reported that when FGFR2IIb was ectopically coexpressed with SOX2, a foregut lineage-survival oncogene in squamous epithelial malignancies, transformation of immortalized tracheobronchial epithelial cells was observed (16). The cooperative transforming effect between GATA6 and FGFR2IIb was assessed using soft-agar assays in both transiently transduced CP-A (p16−/−TP53−/−) Barrett’s cells (Fig. 3A) and in CP-A/FGFR2IIb stable cells that were infected with the GATA6
We next analyzed the growth- and survival-dependency of GATA6. A GATA6 up-regulation was also observed in a subset of dysplastic Barrett’s samples (e.g., 19B). All qRT-PCR reactions were repeated three times. (B) Boxplot analysis of the qRT-PCR data. The y axis represents fold-changes in gene expression relative to the normal intestinal RNA (***P < 0.001, Student’s t test). (C) Immunohistochemistry of GATA6 and MIB1 in EAC TMAs. Overexpression of GATA6 protein was detected in EACs with amplified GATA6 (representative T27, T70, and T83) compared with EAC without GATA6 amplification (T9). (Magnification ×10). MIB1 expression was only observed in tumor T83 that contains MIB1 amplification (Magnification ×20).

GATA6 Sustains EAC Cell Growth and Survival in a Lineage-Specific Manner. We further investigated that GATA6 conferred significantly enhanced anchorage-independent growth in CP-A cells in the presence of FGFR2IIIb (Fig. 3), although the mechanism of interdependency for cellular transformation between GATA6 and FGFR2IIIb is yet to be determined.

There are only a few EAC cell lines available worldwide. The cell lines used in this study, including Flo-1 and OE33 (SI Appendix, Table S5) EAC lines, CP-A and CP-B immortalized Barrett’s cells (20), and Het-A1 and TE13 esophageal squamous cell lines, expressed different levels of GATA6 and none have GATA6 amplification, except for TE13, which has 4–5N copy numbers of GATA6 relative to Flo-1 cells (SI Appendix, Fig. S5 and Table S6). We investigated whether a high level of ectopic expression of GATA6 would alter cell proliferation in cultured EAC cells. When Flo-1 cells that expressed very limited endogenous GATA6 were transduced with GATA6 pBMN6 (SI Appendix, Fig. S5B), a consistent increase in cell proliferation was not observed (SI Appendix, SI Note, and Fig. S6 A–C). However, ectopic expression of GATA6 in cells significantly increased DNA synthesis and S-phase cell cycle distribution in BrdU incorporation assays (SI Appendix, Fig. S6 D and E). A subsequent reduction of S-phase fraction at 48 h following GATA6 transduction was also observed and we speculated that a temporal onset of inducible p21INK4A (21) might account for this observation (SI Appendix, Fig. S6 F and G), indicating the complexity of GATA6 function in these cells.

We further investigated GATA6 modulated tumorigenicity and tumor growth in immune compromised NOD SCID-γ (NSG) mice. Immortalized Barrett’s CP-A cells or EAC FloA cells (FloA cells are derived from Flo-1 cells through soft agar selection) were transiently transduced with expression constructs either pBMN-GATA6 (pBMN6) or pBMN-LacZ (pBMN-Z) and subjected to subcutaneous implantation (SI Appendix, Fig. S7A). Xenograft tumor growth was not observed when 2 × 10^6 CP-A or CP-A/FGFR2IIIb cells with pBMN6 or pBMN-Z were injected in NSG mice for 7 wk. Although most of the tumors that formed at 7 wk in NSG mice after injection with either 5 × 10^6 of FloA, pBMN-Z or FloA/pBMN6 xenografts grew aggressively with significantly large tumors that invaded through adjacent muscle tissue to the abdomen and the bone of the hind leg (SI Appendix, Fig. S7 A–E).

Fig. 2. Overexpression of GATA6 driven by gene amplification in EACs. (A) qRT-PCR analysis of 30 EACs. The y axis shows fold-changes (2-ΔΔCt) of gene expression relative to the normal intestinal tissue (IntN) as GATA6 expression was found to be extremely low or absent in esophageal squamous epithelia (e.g., 43N, and N27 in Q). Overexpression of GATA6 was detected in 13 of 14 EACs containing the GATA6 amplicon. GATA6 up-regulation was also observed in a subset of dysplastic Barrett’s samples (e.g., 19B). All qRT-PCR reactions were repeated three times. (B) Boxplot analysis of the qRT-PCR data. The y axis represents fold-changes in gene expression relative to the expression of normal intestinal RNA (***P < 0.001, Student’s t test). (C) Western blot analysis. Only a small set of primary tissues were examined because of sample availability. Overexpression of GATA6 is shown in a GATA6-amplified EAC (T27) but not in EACs without the GATA6 amplicon (T34 and T78). Samples T27 (EAC), G27 (normal gastric), and N27 (normal esophageal squamous mucosa) were derived from the same patient. (D) Immunohistochemistry of GATA6 and MIB1 in EAC TMAs. Overexpression of GATA6 protein was detected in EACs with amplified GATA6 (representative T27, T70, and T83) compared with EAC without GATA6 amplification (T9). (Magnification ×10). MIB1 expression was only observed in tumor T83 that contains MIB1 amplification (Magnification ×20).

Fig. 3. Colony formation assays following ectopic expression of GATA6. (A) Enhanced cell transformation in immortalized Barrett’s CP-A cells following transient cotransduction of GATA6 and FGFR2IIIb compared with KRAS^C30^ positive control. (B) CP-A/FGFR2IIIb stable cells transduced with GATA6 pBMN6 compared with LacZ control. Significant increase of anchorage-independent growth was observed in CP-A/FGFR2IIIb stable cells transduced with GATA6. Colony count was performed using ImageJ software. qRT-PCR of FGFR2 and GATA6 was used to monitor transduction efficiency (Magnification ×1.5; **P < 0.01, ***P < 0.001, Student’s t test). All transduction and colony formation assays were conducted in triplicate.
was decreased following knockdown of GATA6, with more significant reduction observed in EAC cells than in Barrett’s cells (Fig. 4A and B). In contrast, this reduction of cell proliferation upon knockdown of GATA6 was not found in esophageal squamous cells Het-1A and TE13 (SI Appendix, Fig. 8B). We further observed that siRNA-mediated silencing of GATA6 significantly reduced anchorage-independent growth in OE33 cells (Fig. 4C). Morphological changes characteristic of cell death were observed in EAC cells but not in squamous cells (Fig. 4D). GATA6 silencing induced significant DNA fragmentation indicative of cellular apoptosis in EAC cells (OE33) but not in non-EAC lines (TE13 and Het-1A), as determined by BrdU/TUNEL assays (Fig. 4E-G). To further determine the apoptotic phenotypes induced upon silencing of GATA6, we assessed anoikis, a specific type of apoptosis (22), in Flo-1/GATA6 stable cells. We found that silencing of GATA6 enhanced anoikis as determined by increased poly(ADP-ribose) polymerase (PARP) cleavage, an indicator of caspase 3 activation (Fig. 5A). To validate the effects of siRNA-mediated silencing of GATA6, we performed assays using two additional siRNAs that targeted different coding sequences of GATA6 (SI Appendix, Table S7). PARP cleavage was observed in all three siRNA-treated EAC OE33 cells, but not in squamous TE13 cells (Fig. 5B). In addition, all three siRNA fragments targeting GATA6 significantly increased caspase 3 activity in OE33 cells compared with TE13 cells (Fig. 5C) and produced similar apoptotic phenotypes following GATA6 knockdown (Fig. 5D and SI Appendix, Fig. S9A). Changes in cellular senescence were not found in nonGATA6-expressing EAC Flo-1 cells upon ectopic expression of GATA6 or in siGATA6-transfected OE33 cells using senescence-associated β-galactosidase staining (SI Appendix, Fig. S9B).

Activation of p38α Signaling and Up-Regulation of TNF-Related Apoptosis-Inducing Ligand upon siRNA-Mediated GATA6 Withdrawal in EAC Cells. Because GATA6 is a spatial and temporal master regulator in embryonic development (23) and its deprivation causes massive apoptosis in embryonic ectoderm (24), we speculate that gene amplification-induced differential expression of GATA6 in EAC may cause transcriptional reprogramming in tumor genomes. We used two model EAC cell lines, endogenously limited GATA6-expressing Flo-1 cells and GATA6-expressing OE33 cells (SI Appendix, Fig. S5B). For transcriptional profiling using Affymetrix U133A arrays, Cell RNA was harvested at 24 h to assess an acute response and at 72 h for analysis of the sustained effect of GATA6 regulation following transient transduction of GATA6 (>900-fold) in Flo-1 cells or siRNA-mediated knockdown of GATA6 (>90%) in OE33 cells (SI Appendix, Fig. S10A). Analysis of array data revealed that many genes in diverse cellular pathways were transcriptionally reprogrammed upon differential expression of GATA6 (SI Appendix, Fig. S10B). We were particularly interested in the genes relative to apoptotic pathways following GATA6 silencing in OE33 cells (SI Appendix, Table S8). The proapoptotic genes, TNFSF10/TNF-related apoptosis-inducing ligand (TRAIL), XAF1, and DAPK2, were among the top 100 genes up-regulated upon GATA6-silencing (Fig. 6A and SI Appendix, Table S8), and the results were validated in an
in apoptotic OE33 (p-p38). Both indicates that is a master regulator and amplifications are essential for formation and vol. 109 | siGATA6 | to the esophageal cells under GATA1 modulation in EAC cells induced broad cellular responses. Specific amplifications are indispensable for the result in loss of expression of all hepatocyte nuclear factors in cellular transformation and progression decreased both cell proliferation and in OE33 cells or controls and FloA cells were transduced or pBMN6. OE33* and ** represent two independent experiments. (D) Analyses of caspase 3 (casp3) and cleaved caspase 3 (c-casp3) expression in an apoptosis antibody array (Left) and Western blot of caspase 9 (c-casp9) and cleaved caspase 9 (c-casp9) expression in OE33 cells treated with either siNonTarget control or siGATA6 for 60 h (M, mock). (E) Representative kinase activation from the analysis of 46 phosphorylated kinases in EAC cells with either ectopic expression (FloA) or silencing (OE33) of GATA6 for 60 h using human phosphokinase antibody array. Each phospho-kinase antibody is dotted in doublet. Sample layout is numbered in the upper panel and listed underneath. (F) Western blot analysis of p38α activation. Both OE33 and TE13 cells were treated for 60 h with siGATA6 or controls and FloA cells were transduced with either pBMN-Z or pBMN6 for 60 h. Total p38α and phospho-p38α (p-p38α) were examined and protein extracted from UVC-irradiated Flo-1 cells was used as a positive control. (N, not treated; M, mock).

Discussion
GATA6 is a member of the highly conserved GATA family, which is composed of six zinc-finger transcription factors that regulate lineage-restricted development, differentiation, and cellular aging (27–29). GATA1-3 are essential for formation and differentiation of pluripotent and multipotent hematopoietic stem cells (30), whereas GATA4-6 are indispensable for the lineage-specific development and differentiation of cells of endodermal and mesodermal origin (24, 31). Inactivation of GATA6 in the mouse embryo causes embryonic lethality (24, 32). GATA6 is thought to be a master regulator because inactivation of GATA6 resulted in loss of expression of all hepatocyte nuclear factors in knockout mice (23, 33). In the adult gastrointestinal tract, GATA6 is more localized and expressed within the proliferative and lineage stem-cell zone at the bottom of the gut crypts (34, 35).

Consistent with the idea that GATA6 amplification is a lineage-specific activation, GATA6 amplification was not observed in esophageal squamous carcinoma, as reported in our recent study (16). Interestingly, Kwei et al. (36) and Fu et al. (37) reported that 18q11.2 gain/amplification with overexpression of GATA6 is detected in 9–19% of pancreatic carcinomas. Both the pancreas and distal esophagus are derived from the embryonic endodermal foregut, making it plausible that these two tumors may share common lineage-survival oncogene. Although GATA6 has been reported to be a tumor suppressor in glioblastoma (38) and ovarian (39) cancers, which are tissues of nonendodermal origin, recent comprehensive studies have failed to uncover any evidence for genomic alterations of GATA6 in these diseases (40, 41).

Amplification of lineage-survival oncoproteins imposes survival mechanisms in tumor cells. These factors are otherwise involved in lineage precursor cell development and differentiation (14–17). We hypothesized that GATA6 is a lineage-survival oncogene in EAC based on the fact that GATA6 is a master regulator and stem-cell lineage transcription factor in embryogenesis and that GATA6 amplification is a selective event during the development and progression of EAC. We demonstrated that siRNA-mediated silencing of GATA6 decreased both cell proliferation and anchorage-independent growth in EAC cells and caused a variety of apoptotic phenotypes. The fact that direct tumorigenicity was not affirmed in immortalized Barrett’s CP-A cells indicates that amplification-led overexpression of GATA6 in EAC may impose survival and “stemness” to the esophageal cells under chronic attack from gastro-esophageal reflux and subsequent inflammatory environment, rather than play a role in EAC initiation or formation. We observed that modifying the expression of GATA6 in EAC cells induced broad cellular responses. Specifically, we demonstrated that differential expression of GATA6 caused changes in p38α activation, as well as modulation in the TRAIL-mediated apoptotic pathway. Clearly, further experiments are required to fully understand the oncogenic lineage-survival role of GATA6 in cellular transformation and progression.
of esophageal adenocarcinoma. In light of the lineage-addiction model of human cancer, our present study suggests that thera
depression of GATA6 in GATA6-amplified EAC patients may improve patient survival.

Materials and Methods

Patients and EAC Samples. All animal studies were conducted under the guidelines and approved protocols from the University Committee on Use and Care of Animal of the University of Michigan. Written consent was obtained from each patient according to the approval and guidelines of the University of Michigan institutional review board. Tissues were obtained from patients undergoing esophagectomy for adenocarcinoma at the University of Michigan Health System between 1991 and 2004. Patients in this study had no preoperative radiation or chemotherapy. Specimens were fresh-frozen in liquid nitrogen and stored at −80 °C until use. Cellularity of metaplastic, dysplastic, and tumor samples was assured to be greater than 70% before sample DNA, RNA, or protein was isolated. DNA, RNA, and protein isolation procedures are in SI Appendix, SI Materials and Methods.

Tiling Path Array-CGH and Data Analysis. DNA copy number profiles were generated for 20 EACs using a whole-genome tiling path array, as previously described (42). Data analysis details are in SI Appendix, SI Materials and Methods.

SNP Array Experiments and Analysis. SNP arrays were performed as previously described (16). Briefly, 73 EAC DNAs were genotyped using the Genome-Wide Human St y 120K SNP Array (Affymetrix). Copy number analyses with SNP arrays were performed as a log2 copy number ratio exceeding 0.848 for amplifications and −0.737 for deletions. Genomic positions were mapped in the hg18 genome build. SNP data were visualized using the software IGV 1.3.1 (Integrative Genomics Viewer, www.broadinstitute.org/igv).

Immunohistochemistry of TMAs. Briefly, TMA arrays contained 122 sections from 73 EAC patients, including 63 EAC sections, 18 mixed sections of EAC and dysplasia, 22 Barrett's metaplastic and dysplastic sections, 9 metastatic lymph nodes, and 10 normal sections of various tissue types. Procedure details are in SI Appendix, SI Materials and Methods.

Cell Lines and Culture Conditions. CP-A and CP-B cell lines were kind gifts from Peter Rabinovich (University of Washington, Seattle, WA). CP-A and CP-B were derived from Barrett's metaplasia and high-grade dysplasia, respectively, and were immortalized through induction of hTERT (20). Procedure details are in SI Appendix, SI Materials and Methods.

Statistical Analysis. Kaplan-Meier survival was computed using the GraphPad Prism software and P values were determined by a log-rank test. Box plot analyses were determined using Sigma-Plot software. Analyses in t-test, one-way ANOVA, and correlation coefficient were applied for all necessary experiments.

ACKNOWLEDGMENTS. We thank Dr. Aarif Ahsan for sharing laboratory procedures; Drs. X. X. Xu and C. D. Capo-Chichi for the kind gifts of the pMT-CB6/GATA4 and pMT-CB6/GATA6 constructs. This work was supported by National Cancer Institute Grants R01CA071606-12 (to D.G.B.), K08CA143931 (to A.J.B.), PS0CA90578 (to M.M.), and the University of Michigan Surgery Research Advisory Committee (RAC) Grant (to L.L.).