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ORIGINAL RESEARCH

Gastroesophageal junction adenocarcinoma displays abnormalities in homologous recombination and nucleotide excision repair

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Objective: Esophageal adenocarcinoma (EAC) continues to be a disease associated with high mortality. Among the factors leading to poor outcomes are innate resistance to currently available therapies, advanced stage at diagnosis, and complex biology. Platinum and ionizing radiation form the backbone of treatment for the majority of patients with EAC. Of the multiple processes involved in response to platinum chemotherapy or ionizing radiation, deoxyribonucleic acid (DNA) repair has been a major player in cancer sensitivity to these agents. DNA repair defects have been described in various malignancies. The purpose of this study was to determine whether alterations in DNA repair are present in EAC compared with normal gastroesophageal tissues.

Methods: We analyzed the expression of genes involved in homologous recombination (HR), nonhomologous end-joining, and nucleotide excision repair (NER) pathways in 12 EAC tumor samples with their matched normal counterparts. These pathways were chosen because they are the main pathways involved in the repair of platinum- or ionizing-radiation-induced damage. In addition, abnormalities in these pathways have not been well characterized in EAC.

Results: We identified increased expression of at least one HR gene in eight of the EAC tumor samples. Alterations in the expression of *EME1*, a structure-specific endonuclease involved in HR, were the most prevalent, with messenger (m)RNA overexpression in six of the EAC samples. In addition, all EAC samples revealed decreased expression of at least one of numerous NER genes including *XPC*, *XPA*, *DDB2*, *XPF*, and *XPG*.

Conclusion: Our study identified DNA repair dysregulation in EAC involving two critical pathways, HR and NER, and is the first demonstration of *EME1* upregulation in any cancer. These DNA repair abnormalities have the potential to affect a number of processes such as genomic instability and therapy response, and the consequences of these defects deserve further study in EAC.

Keywords: esophageal adenocarcinoma, DNA repair, MUS81/EME1

Introduction

Esophageal cancer accounts for 4% of cancer deaths in the United States and is the sixth leading cause of cancer deaths among men. Esophageal adenocarcinoma (EAC) incidence has steadily increased during the last 2 decades.[1] In 2013, 17,990 new EAC cases will be diagnosed, and the majority of these individuals have or will succumb to the disease.[2] The rise in the incidence of esophageal cancer is accompanied by a change in the dominant histology of the disease from squamous cell carcinoma of the esophagus to adenocarcinomas mostly diagnosed in the distal esophagus or gastroesophageal junction. A major risk factor for EAC is chronic gastroesophageal reflux disease, which can lead to Barrett's metaplasia, a precursor lesion for EAC.[3] Progression from metaplasia to adenocarcinoma is thought to be mediated by the

development of low-grade followed by high-grade dysplasia (more recently coined intraepithelial neoplasia), yet these histologic abnormalities do not uniformly precede EAC development in all patients.[4]

It has been suggested that all cancers are expected to display a defect in deoxyribonucleic acid (DNA) repair facilitating the accumulation of multiple mutations, which is a shared characteristic of all cancers.[5] DNA repair defects characterizing a number of malignancies are being increasingly identified. For example, homologous recombination (HR) defects were noted in 50% of high-grade serous ovarian tumors.[6] Nucleotide excision repair (NER) deficiency has been previously described in sporadic stage 1 breast cancers, testicular cancers, and lung cancers and has been linked to carcinogenesis and genomic instability.[7–9] Ataxia telangiectasia mutated deficiency has been found in mantle cell lymphomas and gastric cancers.[10,11]

Multiple studies have shown that acid induces DNA damage in the esophagus, and multiple culprits leading to the damage have been described, including nitric oxide, bile acids present in duodenal reflux, and low pH.[12–14] DNA repair pathways involved in the response to damage induced by these agents have not been fully characterized in EAC. Exploring DNA repair abnormalities in esophageal cancer has largely focused on the analysis of DNA repair polymorphisms and their association with cancer susceptibility or therapy response.[15] RAD51, an HR gene, was recently shown to be overexpressed in EAC and was associated with increased genomic instability. Little is known about the expression of other HR genes in addition to NER and nonhomologous end-joining (NHEJ) pathways in EAC. This is the focus of this article.

In this study, we analyzed the DNA repair transcript expression in 12 EAC tumor samples and individually matched normal gastroesophageal tissue. We focused on the HR, NHEJ, and NER pathways, as these are the most relevant to the therapies used in the clinic in the treatment of EAC because of their major role in repair of cisplatin- and/or ionizing-radiation-induced DNA damage. Our data highlight a number of DNA repair alterations that can have a critical effect on both EAC biology and DNA repair.

Materials and methods
Patients and samples
Primary EAC tumor samples and paired normal gastroesophageal tissue of 12 patients who had not received prior chemotherapy or radiation and who underwent esophagectomy at the Indiana University Hospital, Division of Cardiothoracic Surgery, were procured from the Indiana University Melvin and Bren Simon Cancer Center Tissue procurement facility through an institutional review board-approved protocol. All

tumor samples originating from the gastroesophageal area had been snap-frozen immediately on resection in liquid nitrogen and stored until the time of analysis. Consistent with the epidemiology of EAC, all patients except one were men (n=11). The age of the patients ranged from 48 to 83 years, with a median of 74.5 years. Histologic examination was performed by an experienced pathologist on the formalin-fixed paraffin embedded (FFPE) blocks of both normal gastroesophageal and EAC samples to confirm the histology was accurate. Only EAC samples with 70% or more tumors were included in the analysis. Tumor note metastasis (TNM) stage was available for 11 patients, and stage distribution was as follows: one patient had stage 1A carcinoma, four patients had stage 2B disease, five patients were stage 3A, and one patient had stage 3C disease. Patient characteristics are outlined in Table 1.

RNA extraction and complementary DNA synthesis
Total RNA was isolated from 100 mg tissue samples using Trizol (Life Technologies, Carlsbad, CA, USA) and using an RNeasy minikit (Qiagen, Hilden, Germany) per manufacturer's instructions. A nanodrop spectrophotometer was used to determine the quantity of RNA. Complementary (c) DNA was generated using a High Capacity RNA-to-cDNA Kit (Life Technologies, Carlsbad, CA, USA).

Transcript expression measurement by quantitative reverse transcription polymerase chain reaction
The expression levels of 16 reference genes were measured using the Taqman endogenous control array from ABI (catalog no 4367563; Applied Biosystems, Grand Island, NY, USA) to determine the most stably expressed gene between EAC samples and their matched

Table 1 Characteristics of the patients who were included in the analysis

Patient	Age (years)	TNM stage	Grade
1	74	T3N0	Poorly differentiated
2	83	T3N1	Moderately differentiated
3	79	T3N1	Poorly differentiated
4	63	T3N1	Poorly differentiated
5	71	T2N1	Poorly differentiated
6 (female)	48	T1N0	Poorly differentiated
7	65	Unavailable	Unavailable
8	77	T3N0	Poorly differentiated
9	73	T3N1	Moderately differentiated
10	67	T4N1	Moderately differentiated
11	80	T3N0	Poorly differentiated
12	75	T3N1	Poorly differentiated

Abbreviation: TNM, tumor note metastasis.

normal tissue (Figure S1). Seven samples (four tumors and three normal) were included in the endogenous control assay selection. Validated gene primer/probe sets for each gene of interest were purchased from ABI. Taqman reactions were analyzed using ABI's 7900HT system. For each target gene, fold change in expression levels between normal and tumor sample was evaluated using ABI's ∆∆CT Relative Quantification methodology. Data were normalized to the housekeeping gene *GUSB* (glucuronidase, beta) (Hs99999908_m1), and each tumor sample was calibrated to its normal matched control. Results presented are the average of two quantitative reverse transcription polymerase chain reaction (qRT-PCR) analyses with triplicate Taqman assays per RNA sample set. Genes analyzed and assays used are outlined in Table 2.

Statistical analysis

Statistical analysis was performed using a paired Student's two-tailed *t*-test (SigmaStat, Systat, Point Richmond, CA,

Table 2 Summary of DNA repair genes analyzed with their average fold changes and *P*-values

Pathway and gene symbol	Assay	Average fold change	*P*-value	Number of tumors with alterations
Nucleotide excision repair				
XPA	Hs00166045_m1	0.48	0.0004*	12↓
DDB1	Hs00172410_m1	0.92	0.207	5↓
DDB2	Hs03044953_m1	0.75	0.0435*	8↓
ERCC1	Hs01012158_m1	0.76	0.0644	9↓
ERCC2	Hs00361161_m1	1.15	0.162	2↓
ERCC3	Hs01554450_m1	1.08	0.478	1↓
XPF	Hs00193342_m1	0.710	0.004*	7↓
XPG	Hs00164482_m1	0.715	0.039*	10↓
XPC	Hs01104206_m1	0.633	0.009*	9↓
RPA1	Hs00161419_m1	0.900	0.234	6↓
RPA2	Hs00358315_m1	0.996	0.535	5↓
Homologous recombination				
RAD51	Hs00153418_m1	3.039	0.073	5↑
RAD52	Hs00172536_m1	1.218	0.254	2↑
BRCA1	Hs01556193_m1	1.867	0.187	5↑
BRCA2	Hs00609073_m1	1.867	0.137	5↑
EME1	Hs00385890_m1	2.971	0.007*	6↑
MUS81	Hs00228383_m1	0.833	0.457	4↓
Nonhomologous end-joining				
Artemis	Hs01052780_m1	0.98	0.127	6↓
DNAPK	Hs01016093_m1	1.49	0.485	2↓
XRCC5	Hs00221707_m1	1.00	0.602	5↓
XRCC6	Hs01922652_g1	0.86	0.267	4↓
ATM	Hs01112307_m1	0.98	0.467	5↓
ATR	Hs00354807_m1	1.37	0.652	2↓
Ligase IV	Hs00172455_m1	1.28	0.678	3↓

Note: *Statistically significant genes, *P*≤0.05.
Abbreviation: DNA, deoxyribonucleic acid.

USA). Differences between groups at *P*≤0.05 were considered statistically significant.

Immunohistochemistry

FFPE tissue sections corresponding to the EAC tumor samples were available for nine of the 12 samples. Immunohistochemistry (IHC) was performed with the assistance of the IHC pathology laboratory at Indiana University. FFPE blocks were cut into 4 µm thick sections and collected on charged slides, and immunostaining was performed using the Autostainer Plus platform from Dako (Carpinteria, CA, USA). Slides were heated at 65°C for 30 minutes, deparaffinized with xylene, and rehydrated with graded alcohol. Endogenous peroxidase activity was blocked using 3% peroxidase blocking reagent (Dako K8002) for 5 minutes. Antigen retrieval was performed by immersing sections in Dako's PT module filled with FLEX Target Retrieval Solution, low pH (K800521), for 20 minutes. Sections were incubated for 60 minutes with a 1:10 dilution of EME1 primary antibody (MTA31 7h2/1) from Santa Cruz Biotechnology (Dallas, TX, USA; sc-53275). Detection was performed using an Envision FLEX+ mouse kit from Dako. Normal uterine tissue was used as a positive control to optimize IHC conditions. All sections were examined by a surgical pathologist who confirmed the histology of normal/nonneoplastic esophageal tissue and EAC samples by reviewing hematoxylin and eosin sections. IHC staining for EME1 was scored on the basis of the intensity of nuclear staining (0, absent; 1, faint; 2, moderate; and 3, strong staining) and the extent of staining (0, 0%; 1, 1%–10%; 2, 10%–50%; and 3, >50% of tumor cells staining).

Western blot

Western blots were used to ensure specificity of the primary EME1 antibody, as this is the first report of the assessment of EME1 expression by IHC. HeLa cell, WI-38 (normal lung fibroblasts), and SK-GT4 (esophageal adenocarcinoma) cell line extracts were prepared as previously described.[16] Protein concentration was determined using the Bio-Rad protein assay system (Bio-Rad Laboratories, Hercules, CA, USA) with bovine serum albumin standards. Forty micrograms of protein were loaded onto sodium dodecyl sulfate polyacrylamide gel electrophoresis, and after electrophoresis, proteins were transferred to polyvinylidene difluoride membranes. EME1 protein was detected using EME1 antibody (MTA31 7h2/1) at a 1:1000 ratio, with secondary immunoglobulin (Ig)G horseradish peroxidase anti-mouse antibody (sc-2005, Santa Cruz) at a 1:4000 dilution.

Oncomine database search

The Oncomine Cancer Profiling Database version 4.4.3, September 2012 data release (http://www.oncomine.org; Life Technologies, Carlsbad, CA, USA), was used to profile the gene *EME1*. Expression data and statistics were obtained directly through the Oncomine 4.4.3 software.

Results

EAC shows messenger RNA overexpression of HR genes

Defects in a number of HR genes have been described in solid organ malignancies and have been shown to mediate sensitivity to a variety of chemotherapy agents including platinum, crosslinking agents, and poly(ADP-ribose) polymerase inhibitors.[17–19] We initiated our work by screening 16 endogenous housekeeping genes to determine the optimal endogenous control and identified *GUSB* as an excellent housekeeping gene with small differences in expression between tumor and normal tissues (Figure S1). All genes of interest were therefore normalized to *GUSB* transcript levels, and each tumor sample was compared with its matched normal sample. Eight of the 12 EACs showed messenger (m) RNA overexpression of at least one of the HR genes, including *RAD51*, *RAD52*, *BRCA1*, *BRCA2*, and *EME1* (twofold cutoff or greater; Figure 1). *EME1* was overexpressed in six of the 12 EACs, and the difference in expression was statistically significant compared with normal tissue ($P<0.001$).
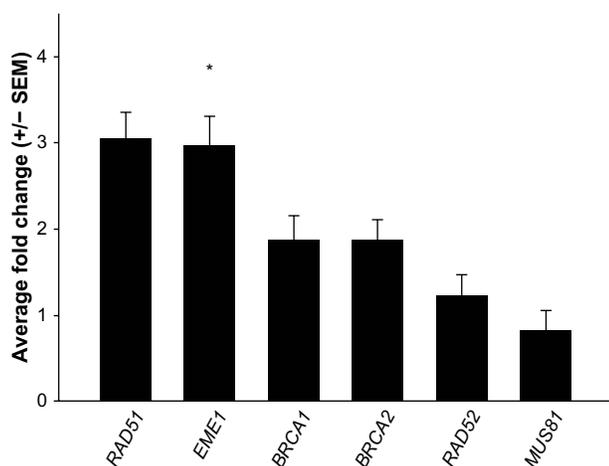

Figure 1 Quantitative reverse transcription polymerase chain reaction analyses of messenger (m)RNA expression changes of homologous recombination genes. Data shown are the average of two separate quantitative reverse transcription polymerase chain reaction assays. Genes of interest were normalized to *GUSB* transcript levels and are presented as fold changes relative to normal matched samples. *EME1* transcripts are increased in EAC samples.
Notes: *Statistically significant results with *P*-value ≤ 0.05.
Abbreviations: SEM, standard error of the mean; EAC, esophageal adenocarcinoma.
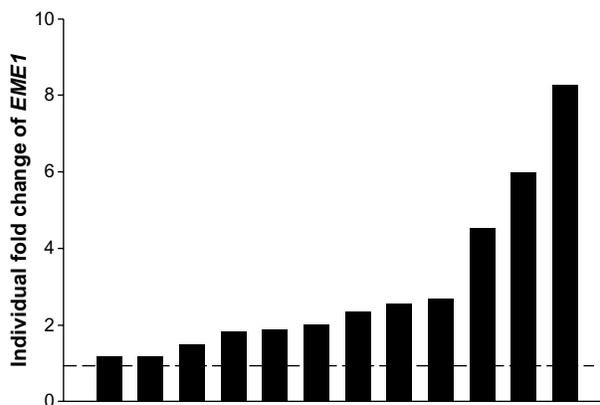

Figure 2 *EME1* messenger (m)RNA expression in individual esophageal adenocarcinoma samples, showing three tumor samples display more than fourfold overexpression of *EME1*.
Note: Dotted line refers to fold change of 1.

Three of these tumor samples showed greater than fourfold *EME1* mRNA overexpression (Figure 2).

The MUS81/EME1 protein complex is an endonuclease in the HR pathway with cleaving DNA intermediates as its main function. It plays an important role in maintaining genomic integrity, yet its role in human disease and cancer is still undefined. As EME1 is present in complex with MUS81, we analyzed mRNA expression of *MUS81* in the EAC samples and found that four tumor samples showed underexpression of *MUS81* when compared with a matched normal sample. *RAD51* overexpression approached statistical significance with a threefold change in EAC samples compared with normal counterparts. NHEJ genes evaluated showed a slight reduction in transcript expression in a few EAC samples when compared with normal counterparts, but none of them were statistically significant (Table 2). Three of the patients had two samples from separate areas of the tumor available, and with the recent data revealing tumor heterogeneity in drug target expression and genomic differences within the same renal cell carcinoma sample, we were interested in whether we would be able to identify the heterogeneity of *EME1* expression in different areas of the tumor.[20] Transcript expression of the HR genes was reproducible between these biological duplicates (Table S1), suggesting EAC may not be as heterogeneous as renal cell carcinoma. We observed that all HR genes followed similar patterns in each tumor sample, where the majority of them either showed increased transcript expression or no change. Table 2 outlines genes evaluated in this analysis, along with fold change results.

EME1 overexpression in EAC by IHC

As this is the first published report of *EME1* upregulation in cancer, we were interested in assessing whether changes in

the mRNA expression of *MUS81/EME1* are accompanied by changes in its protein expression. Therefore, EME1 IHC was performed on FFPE sections corresponding to the fresh frozen samples analyzed by qRT-PCR for mRNA expression. Western blot assessment confirmed that the commercially available EME1 antibody used in these analyses did not show cross-reactivity and was highly specific for EME1, showing one dominant band at the expected size of 65 kDa noted in the HeLa and SK-GT4 cell lines, both of which represent positive controls (Figure S2). The WI-38 cell line, in contrast, does not display increased expression of EME1 with a weaker band observed.

Normal uterine tissue was used as a control to optimize IHC conditions and showed an appropriate nuclear staining pattern of EME1 (Figure 3, insert). IHC analysis revealed that EME1 was overexpressed at varying intensities in nine of nine tumor samples available for testing compared with normal tissues (Figure 3). Of note, EME1 staining intensity was noticeably less evident in the less-differentiated areas of the tumor, but the significance of this observation is unclear. Of note, gene and protein expression of EME1 did not necessarily correlate, as adenocarcinoma samples numbered 1, 2, and 11 showed less than twofold increase in *EME1* mRNA expression but moderately increased EME1 IHC staining, and tumor sample 10 had intense IHC staining that was not associated with any change in *EME1* mRNA expression (Table S2).

EACs display defects in NER

The NER pathway plays a significant role in repairing a variety of DNA lesions, including those resulting from ultraviolet radiation, chemotherapy agents, and mutagens.[21] Our analysis of the NER pathway showed that each of the 12 tumor samples displayed an abnormality in transcript expression of at least one of the eleven NER genes analyzed (Table 2). *XPC*, *XPA*, *DDB2*, *XPF*, and *XPG* genes were statistically significantly underexpressed in EACs compared
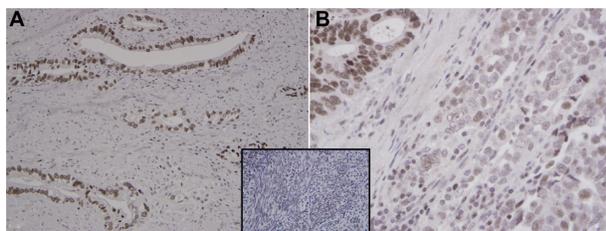

Figure 3 EME1 immunohistochemistry in gastroesophageal junction adenocarcinoma. (**A**) Well-differentiated gastroesophageal junction adenocarcinoma shows uniform strong nuclear staining pattern with EME1 (250×). (**B**) EME1 staining intensity was noticeably less in the less differentiated areas (500×). Note the nuclear immunoreactivity for EME1 in a control of benign endometrial stroma (inset).

with normal samples (Table 2). *XPA* was underexpressed in all 12 EAC samples, with fold changes in the range of 0.2 to 0.7, and this was statistically significant across all samples. This finding is consistent with the increased incidence of cancer in patients with xeroderma pigmentosum.[22] The observation of NER deficiency in all EAC samples analyzed is extremely intriguing, especially with the role of this pathway in repair of DNA damage resulting from various sources.

Underexpression of NER components has been tied to poor prognosis in cancer, with clinical data suggesting it is more likely observed in higher stages of cancer as compared with earlier stages.[23] As only one patient had stage 1 disease and still displayed decreased expression of NER components, it is unclear whether alterations in NER expression are more frequently encountered in more advanced EAC disease stages. It is potentially possible, however, that underexpression of NER in EAC contributes to its unfavorable prognosis.

EME1 is overexpressed in a number of cancers

EME1 overexpression noted in EAC in this study has not been previously described. In fact, *EME1* overexpression has not been published in any cancer. This led us to conduct a search of the Oncomine gene expression database to evaluate whether other tumors overexpress *EME1*, and we chose a twofold change as threshold. This search showed that *EME1* is overexpressed in head and neck, breast, and central nervous system tumors when compared with normal tissues. In addition, in one data set, papillary renal cell carcinoma and lymphoma each displayed mRNA overexpression of *EME1* (Table 3). There are a limited number of data sets for EAC in the Oncomine database, but our search did show *EME1* was overexpressed in a portion of EACs compared with normal esophageal tissue in the Kim Esophagus data set. *EME1* seemed to be more highly expressed in our EAC samples when compared with data sets of other tumors reported in Oncomine, where the fold change was between 2 and 3. This observation might suggest that *EME1* upregulation in EAC is unique, possibly as a response to acid damage.

Discussion

Multiple studies have highlighted genetic alterations in EAC, revealing mutations in the frequently mutated genes such as *TP53* and *PIK3CA*, as well as less common mutations in *SPG20*, *TLR4*, *ELMO1*, and *DOCK2*.[24,25] Limited data exist describing the DNA repair alterations present in EAC and with the fundamental role of chemotherapy and radiation in this disease, understanding those alterations has the potential

Table 3 Summary of Oncomine database search results of tumors with increased *EME1* expression

Data set and cancer type	Number of samples	*EME1* expression fold change	*P*-value
French brain			
Anaplastic oligoastrocytoma	33	2.615	8.5E–5
Oligodendroglioma	33	2.3	8.24E–7
Murat brain			
Glioblastoma	84	2.968	1.31E–7
Richardson breast 2			
Ductal carcinoma	47	2.770	2.62E–9
TCGA breast			
Invasive ductal carcinoma	47	2.165	2.08E–27
Invasive lobular carcinoma	47	2.071	1.71E–8
Pyeon multicarcinoma			
Tonsillar carcinoma	84	2.162	4.28E–5
Ye head-neck			
Tongue squamous cell carcinoma	38	2.172	3.02E–6
TCGA renal			
Papillary renal cell carcinoma	88	2.380	3.06E–7
Compagno lymphoma			
Diffuse large B cell lymphoma	136	2.459	1.50E–14

Note: Data available from: http://www.oncomine.org.[34]
Abbreviation: TCGA, the cancer genome atlas.

of further refining therapies and improving patient outcomes. In this study, we performed gene expression analysis of a focused group of DNA repair genes in EAC samples arising in the gastroesophageal junction area. The genes were carefully selected because of their involvement in the repair of damage induced by platinum or ionizing radiation in addition to existing data showing alterations in these genes in other malignancies. Our findings highlight a number of important observations including NER deficiency and HR overexpression in EAC.

Our analysis of 12 EAC samples shows that alterations in transcript expression of at least one NER gene are detected in all tumor samples, with *XPC*, *XPA*, *DDB2*, *XPF*, and *XPG* showing significant underexpression in tumors compared with their normal counterparts. Defective NER, through reduced expression of some of its components, especially *XPA* and *ERCC1*, has been postulated to be one of the main mechanisms of hypersensitivity of testicular cancer, including in the metastatic stage to cisplatin therapy.[26] There is an abundance of data demonstrating the connection between platinum resistance and NER function. *XPA* is an essential NER component and a rate-limiting factor in excision repair, and its decreased expression in all EAC samples evaluated would suggest potential EAC sensitivity to cisplatin.

However, clinical data are not consistent with that, as cisplatin has modest activity in EAC and, as a single agent, has response rates of only 20%.[27] The patients included in this analysis were not candidates for chemotherapy, and we are therefore unable to assess the effect of NER alterations via underexpression of *XPA* on their response to platinum-based therapy. However, on the basis of outcomes of patients with T3N1 EAC (the same stage as nine of 12 patients included in this analysis), estimated cure rates with the combination of platinum-based chemotherapy, radiation, and surgery are only 50%.[28] These observations highlight the complexity of predicting response to platinum, which is orchestrated by multiple steps and proteins in the NER pathway in addition to other pathways such as HR playing a role, making it doubtful that the expression of a single DNA repair factor will dictate response to platinum.

Another finding in our analysis is that two thirds of EACs display increased expression of at least one of the HR genes analyzed, specifically *BRCA1*, *BRCA2*, *RAD51*, *RAD52*, and *EME1*. The HR pathway is mainly involved in the repair of DNA double strand breaks, yet consistently, HR defects have been shown to increase sensitivity to a wide variety of chemotherapy agents, including cisplatin.[29] This more general effect of HR on therapy response could be related to the many proteins and subpathways involved in HR. The HR pathway is critical for the maintenance of genomic integrity, and further studies are needed to assess the consequences of upregulation of HR in EAC described here on the genomic instability noted in EAC. We have demonstrated no change in expression of the NHEJ genes evaluated; however, mRNA expression alone does not reflect the function of the NHEJ pathway in EAC. Both NHEJ and HR activity are regulated by protein interactions and posttranslational modifications, rather than transcription alone. The efficiency of NHEJ renders it less affected by transcription alone, and therefore, normal NHEJ gene expression does not necessarily mean normal function. Potential limitations of our study include the relatively small sample size.

Of the genes analyzed in this study, *EME1*, a component of the structure-specific endonuclease complex MUS81/EME1, demonstrated the most frequent and statistically significant overexpression in EAC. *EME1* shows increased transcript expression in 50% of EACs, with three tumor samples showing more than fourfold overexpression. In addition, EME1 protein expression is increased at varying intensities when analyzed by IHC in all EAC samples. Our study illustrates that although *EME1* shows increased expression in EAC, its partner *MUS81* showed either decreased

or unchanged mRNA expression. We are unaware of other reports evaluating the mRNA expression of both components of the *MUS81/EME1* complex, but in another study, correlation patterns of EME1 and MUS81 protein levels in vitro varied according to the cell lines analyzed.[30] In addition, our data demonstrate that *EME1* mRNA levels do not correlate with the protein expression absolutely (Table S1). These observations lead to a number of possibilities, including increased EME1 protein stability or posttranscriptional regulation of EME1 in some tumors.

A variety of biochemical and structural aspects of the MUS81/EME1 complex have been elucidated during the last decade, but its role in carcinogenesis remains to be defined. *MUS81/EME1*'s contribution to the recovery of stalled replication forks, a crucial step in preserving genome stability, suggests *MUS81/EME1* defects would possibly mediate genomic instability, a bona fide hallmark of cancer.[31,32] This is supported by a number of observations including the associated increase of spontaneous gross chromosomal rearrangements in the setting of defective *MUS81* or *EME1*.[33] Our search of the Oncomine Database showed that increased *EME1* mRNA overexpression is not limited to EAC, as a number of malignancies display two- to threefold increased expression of *EME1* (Table 3).

In conclusion, our study highlights a number of alterations in the DNA repair expression profile of EACs, including NER deficiency, HR upregulation, and the novel finding of increased expression of *EME1*. These defects possibly contribute to the genomic instability of EAC and potentially mediate resistance to chemotherapy and radiation that remains a challenge in caring for these patients. Discovering the implications of DNA repair abnormalities in EAC carcinogenesis and therapy response has the potential to improve outcomes of EAC. We are currently pursuing further studies to dissect the role of *EME1* overexpression in EAC pathogenesis.

Acknowledgment

Disclosure
The authors report no conflicts of interest in this work.

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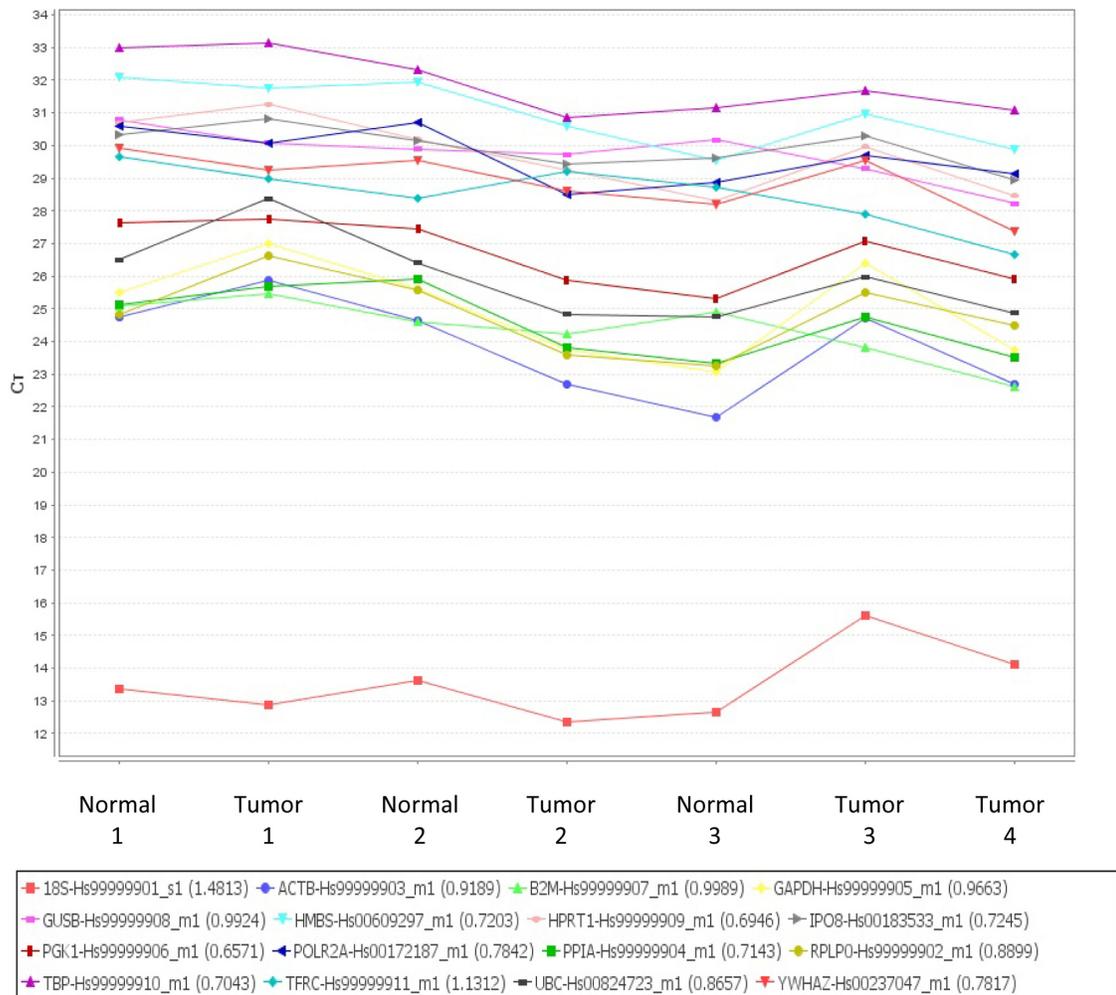
Supplementary materials

Figure S1 Taqman endogenous control assay used to determine the optimal housekeeping gene. X axis shows samples used, Y axis showed Ct values of each gene.
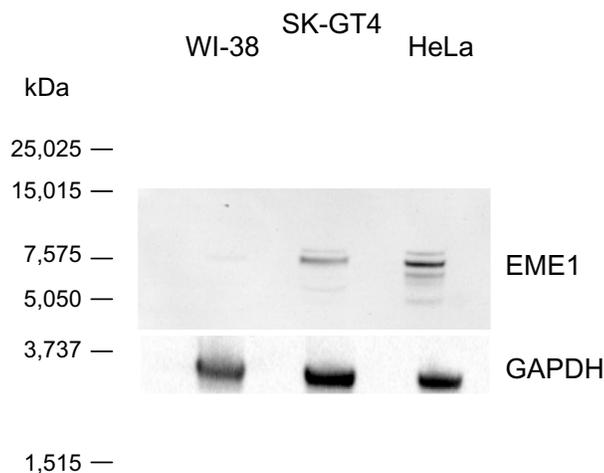

Figure S2 Western blot analysis evaluating EME1 primary antibody in HeLa, WI-38, and SK-GT4 cell extracts. One major band can be seen at expected size of 65 kDa in both HeLa and SK-GT4 cell extracts. The band is present but much weaker in the WI-38 cell line.
Abbreviation: GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Table S1 Summary of messenger RNA expression of homologous recombination genes in three tumor samples and their biological duplicates

Gene and tumor number	Average fold change	Biological replicate	Average fold change
EME1			
4	4.5	T4 replicate	5.7
3	5.9	T3 replicate	3.3
2	1.9	T2 replicate	0.9
RAD51			
4	7.6	T4 replicate	12.8
3	1.5	T3 replicate	1.5
2	0.5	T2 replicate	0.5
RAD52			
4	0.7	T4 replicate	0.5
3	2.3	T3 replicate	2.1
2	0.4	T2 replicate	0.4
BRCA1			
4	3.1	T4 replicate	1.5
3	5.2	T3 replicate	2.9
2	0.7	T2 replicate	0.5
BRCA2			
4	4.2	T4 replicate	5.4
3	3.7	T3 replicate	1.7
2	0.8	T2 replicate	0.8
MUS81			
4	0.8	T4 replicate	0.4
3	1.0	T3 replicate	1.0
2	0.3	T2 replicate	0.1

Table S2 Summary of results of *EME1* messenger (m)RNA and EME1 protein expression in each of the individual esophageal adenocarcinoma samples

Sample number	EME1 immunohistochemistry score	*EME1* mRNA fold change
1	2+2+	1.8
2	2+2+	1.9
3	2+3+	5.9
4	2+2+	4.5
5	Block not available	1.4
6	Block not available	1.1
7	3+3+	2.3
8	Block not available	2.5
9	2+3+	2.6
10	3+3+	1.1
11	3+2+	1.8
12	2+3+	8.2