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Specific gene expression profiles are associated with a pathologic complete response to neoadjuvant therapy in esophageal adenocarcinoma

Patrick J. McLaren, MD [a], Anthony P. Barnes, PhD [b], Willy Z. Terrell, BS [a], Gina M. Vaccaro, MD [c], Jack Wiedrick, MS [d], John G. Hunter, MD [a], James P. Dolan, MD, MCR [a, *]

[a] Department of Surgery, Division of Gastrointestinal and General Surgery, Oregon Health and Science University, Portland, OR, USA
[b] Knight Cardiovascular Institute, Department of Medicine, Oregon Health & Science University, Portland, OR, USA
[c] Department of Medicine, Division of Medical Oncology, Oregon Health and Science University, Portland, OR, USA
[d] School of Public Health, Division of Biostatistics, Oregon Health and Science University, Portland, OR, USA

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ABSTRACT

Background: Predicting treatment response to chemo-radiotherapy (CRT) in esophageal cancer remains an unrealized goal despite studies linking constellations of genes to prognosis. We aimed to determine if specific expression profiles are associated with pathologic complete response (pCR) after neoadjuvant CRT.

Methods: Eleven genes previously associated with esophageal cancer prognosis were identified. Esophageal adenocarcinoma (EAC) patients treated with neoadjuvant CRT and esophagectomy were included. Patients were classified into two groups: pCR and no-or-incomplete response (NR). Polymerase chain reaction was used to evaluate gene expression. Omnibus testing was applied to overall gene expression differences between groups, and log-rank tests compared individual genes.

Results: Eleven pCR and eighteen NR patients were analyzed. Combined expression profiles were significantly different between pCR and NR groups ($p < 0.01$). The gene CCL28 was over-expressed in pCR patients (Log-HR: 1.53, 95%CI: 0.46–2.59, $p = 0.005$), and DKK3 was under-expressed in pCR (Log-HR: −1.03 95%CI: −1.97, −0.10, $p = 0.031$).

Conclusion: EAC tumors that demonstrated a pCR have genetic profiles that are significantly different from typical NR profiles. The genes CCL28 and DKK3 are potential predictors of treatment response.

1. Introduction

Even with optimal treatment, the median 5-year survival for patients diagnosed with esophageal cancer remains less than 50%.[1] Current multimodality regimens often carry significant risk despite advancements in therapy. It is estimated that 3% of patients treated with neoadjuvant chemoradiotherapy (CRT) will die before surgery, and 37% will suffer grade 3 or worse toxicity when undergoing treatment.[2] Subsequent esophagectomy has an operative mortality rate ranging from 3 to 12% and up to 50% morbidity.[3–6] Given these data, it is clear that any improvements in current treatment regimens would be welcomed in the face of an alarming increase in the incidence of esophageal adenocarcinoma (EAC) in the United States.[7,8]

Clinical trials have demonstrated that CRT followed by esophagectomy has a survival benefit compared to surgery alone. This has led to the adoption of trimodality therapy as standard treatment for loco-regional disease.[9] Unfortunately, individual treatment response to neoadjuvant therapy is highly variable. Between 25 and 30% of esophageal cancer patients will have no residual malignant cells present on final pathologic exam, termed a pathologic complete response (pCR).[9,10] More importantly, it has been demonstrated that achieving a pCR to neoadjuvant therapy portends significantly improved survival.[11] Thus, novel treatments targeted at improving pCR rate have the potential to significantly improve

* Corresponding author. Mail Code L223A, 3181 SE Sam Jackson Park Rd., Portland, OR, 97239, USA.
E-mail address: dolanj@ohsu.edu (J.P. Dolan).

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outcomes.

Many studies have attempted to identify histopathologic markers associated with pCR, but the data regarding such markers has been inconclusive.[12–14] A possible explanation for the heterogeneity in treatment response may be explained by differing gene expression profiles of the individual tumors. Evaluating tumor genetics has proven useful in other cancers to assess likelihood of treatment response.[15] These technologies are now being used clinically to individualize treatment plans, and provide patients and clinicians with prognostic information. Similar approaches to esophageal cancer have not yet been proven to provide consistent and definite predictive power in the clinical setting. Prognosis prediction in EAC remains an unrealized goal despite rapid growth in the fields of genomics and proteomics. Our hope in identifying clear prognostic indicators is that clinicians can reliably predict treatment response, then individualized treatment plans can be developed to minimize adverse effects while providing the best possible chance at a cure. The aim of this study was to evaluate existing candidate genes using our EAC tissue repository for association with a favorable treatment response in the context of current standard of care neoadjuvant CRT.

2. Methods

2.1. Patient selection

This study was approved by the Oregon Health and Science University (OHSU) IRB (#1759). The OHSU Esophageal Cancer and Related Diseases database (ECRD) is a prospectively maintained registry of clinical and pathologic data on all patients treated with esophageal cancer at our institution. We identified patients from the ECRD with the diagnosis of EAC treated with neoadjuvant CRT and esophagectomy between January 2011 and July 2015. Given our focus on EAC, patients with squamous cell carcinoma (SCC) histology were excluded. EAC patients with pretreatment formalin-fixed, paraffin-embedded (FFPE) esophageal biopsies in our tissue repository were included. Medical records were reviewed for pathologic classification based on final surgical pathology as evaluated an expert pathologist within the OHSU department of surgical pathology. A pCR was defined as no evidence of tumor on final pathologic exam in the primary specimen or associated lymph nodes. Any evidence of residual tumor cells was classified as non-or-incomplete responder (NR), regardless of down staging or degree of primary tumor reduction.

2.2. Gene selection, RNA isolation, and qPCR

Candidate genes for investigation were identified based on previous publications and our own preliminary studies. Eleven genes were selected for investigation in our cohort due to their association with esophageal cancer prognosis.[16–21] The target genes investigated were CCL28,[20] SPARC,[18] S100A2,[19] SPRR3,[19] SIRT2,[21] NOV,[20] PERP,[19] PAPSS2,[21] DCK,[21] DKK3,[22] ALDH1.[16] The gene ACTB was selected as the endogenous control based on published work.[17]

Two ten-micron sections were taken from each specimen block and RNA was isolated from FFPE tissue using the RNeasy Mini Kit FFPE (Qiagen). Following RNA isolation, RNA quality assessment was performed using the Agilent 2100 Bioanalyzer with a Eukaryote total RNA Nano chip. Reverse transcription was performed using the SuperScript VILO cDNA synthesis kit (Life Technologies) with 100 ng of input RNA per 20 μl reaction. Following reverse transcription, 200 ng of cDNA was used in a pre-amplification reaction using the TaqMan pre-amplification master mix (Life Technologies) which included a pool of all 12 primer/probe sets at a 0.2X concentration. The qPCR assays were performed on the QuantStudio RealTime PCR System (Life Technologies) using TaqMan probes for the eleven target genes and ACTB. Data was collected using Applied Biosystems QuantStudio™ 12 K Flex Software v1.0.

2.3. Statistical analysis of gene expression

Cycle threshold (Ct) values were used as a measure of gene expression level, with lower values representing higher levels of gene expression. Individual sample variation in Ct values for the control, ACTB, was normalized using regression models to adjust for mean expression across all genes. The normalized Ct values of the target genes were interpreted as expression relative to ACTB. The gene expression profiles of pCR versus NR groups were then compared in order to identify differential expression patterns between groups. An omnibus test using Mahalanobis distance from the multigene centroid of the NR group was applied to evaluate overall gene expression differences between pCR and NR. Log-rank tests were applied to compare the differential expression of individual genes between groups, and comparisons made based on hazard ratios. Statistical significance was set at a p-value of 0.05. Statistical analysis was carried out using STATA statistical software (Version 14, College Station, TX, USA).

3. Results

From January 2011 to July 2015, 29 patients had pre-treatment biopsy specimens available within our FFPE tissue bank for inclusion in the study. The average age of the patient population was 67.7 years and ranged from 56 to 79 years. There was only one female (3%) in the cohort, and the entire sample population was Caucasian. Clinical stage of disease ranged from IB to IIIB. Of the 29 patients, eleven (38%) were identified as having a pCR to neoadjuvant therapy, and eighteen (62%) were classified as NR with residual tumor on final pathology. There were no statistically significant differences between groups with respect to age at diagnosis, race, gender, clinical stage, or chemotherapy regimen. Baseline characteristics are shown in Table 1. All pCR specimens had significantly different combined genetic profiles versus the prototypical NR, comparing the combined genetic profiles between groups (Fig. 1). This is demonstrated by the fact that all specimens from patients with a pCR have a Mahalanobis distance greater than the 99th percentile of the estimated distance distribution of the NR samples. Expression levels between groups for each individual gene is shown in Fig. 2. CCL28 was over-expressed in pCR (Log-HR: 1.53, 95%CI: 0.46−2.59, $p = 0.005$). Expression of CCL28 was increased by a factor of 2.28 in pCR specimens. Conversely, DKK3 was under-expressed in pCR tumors (Log-HR: −1.03 95%CI: −1.97, −0.10, $p = 0.031$). Expression of DKK3 in pCR specimens was 0.85 times that of the NR group. None of the other nine target genes demonstrated statistically significant differences between groups (Table 2).

4. Discussion

Outcomes in patients treated with multimodality treatment for EAC are highly variable. Even when matched for stage and demographics, patients receiving the same treatment can have vastly different outcomes. Early identification of treatment response group could aid in determining the best treatment plans in order to avoid over- or undertreating. Unfortunately, there is currently no method to reliably predict which patients will manifest a pCR prior to undergoing an esophagectomy. Because of the difficulty in identifying pCR prior to surgery, esophagectomy is recommended