Original Article

A Validated miRNA Profile Predicts Response to Therapy in Esophageal Adenocarcinoma

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BACKGROUND: In the current study we present a validated miRNA signature to predict pathologic complete response (pCR) to neoadjuvant chemoradiation in esophageal adenocarcinoma. **METHODS:** Three patient cohorts (discovery, n = 10; model, n = 43; and validation, n = 65) with locally advanced esophageal adenocarcinoma were analyzed. In the discovery cohort 754 miRNAs were examined in pretreatment tumor biopsy specimens using a TaqMan array. Of these, the 44 most significantly altered between tumors with pCR and non-pCR were examined in an additional 43 tumors using a Fluidigm 48.48 array. The 4 miRNAs (mir-505*, mir-99b, mir-451, and mir-145*) significantly predicting pCR in both cohorts were examined in an additional validation cohort (n = 65) using an Illumina array. These 4 miRNAs were used to generate an miRNA expression profile (MEP) score. **RESULTS:** The 4 miRNAs profiled are highly significantly associated with pCR in the model cohort ($P_{trend} = .008$), the validation cohort ($P_{trend} = .025$), and the combined cohort ($P_{trend} = 4.6 \times 10^{-4}$). The receiver-operator characteristic areas under the curves (AUCs) for the MEP score were 0.78 for the model cohort, 0.71 for the validation cohort, and 0.72 for the combined cohort. When combined with clinical variables, the MEP score AUCs increased to 0.89, 0.77, and 0.81, respectively Estimates from logistic regression based on the MEP were determined and used to generate a probability of pCR plot, which identifies a group of patients with very high (≥80%) and very low (≤10%) probability of pCR. **CONCLUSIONS:** The MEP score provides a validated means of predicting pCR to neoadjuvant chemoradiotherapy in esophageal adenocarcinoma that is robust across several analysis platforms. ***Cancer* 2014;120:3635-41.**

KEYWORDS: miRNA, esophageal cancer, response, chemotherapy, radiation.

INTRODUCTION

The incidence of esophageal adenocarcinoma is on the rise in the United States and Europe, with the majority of patients diagnosed with locally advanced disease.[1] Although treatment strategies vary, in many instances, patients are treated with preoperative chemoradiotherapy followed by surgical resection. This approach has been validated recently in a randomized trial, with improved survival using neoadjuvant chemoradiation followed by surgical resection compared with surgery alone.[2] Based on this trial and others, pathologic complete response (pCR) rates around 25%-30% are expected after this therapy.[3] Because of the significant morbidity of surgical resection, definitive chemoradiotherapy alone has also been explored.[4] Recently a phase II trial investigated this approach in patients with complete response assessed by endoscopic and/or imaging methods, with the encouraging outcome of a 1-year survival rate of 71%.[5] However, close to 50% of these patients eventually required surgical salvage within the relatively short follow-up period. This inability of clinical methods to accurately predict response to chemoradiation in esophageal adenocarcinoma has been demonstrated elsewhere.[6-8] Thus, the ability to practice a selective surgical approach is severely hampered. In addition, a significant proportion of patients' tumors are highly resistant to standard chemoradiotherapy and may progress during treatment.[9] This group of patients may be best served with some combination of upfront surgical resection and enrollment in a clinical trial. However, at present no method exists to predict a priori with any reasonable degree of accuracy the sensitivity to therapy of any particular esophageal adenocarcinoma.

The most promising approach to predicting response to therapy in this disease likely involves a combination of biomarkers that would be easily quantifiable, translatable across multiple assessment platforms, and, most importantly,

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TABLE 1. Patient and Tumor Characteristics of the Study Population

	Discovery Set	Model Set	Validation Set
N	10	43	65
Sex, n (%)			
Male	9 (90)	40 (93)	63 (96.9)
Female	1 (10)	3 (7)	2 (3.1)
Stage, n (%)			
II	5 (50)	16 (37.2)	27 (41.5)
III-IV	3 (30)	24 (55.8)	29 (44.6)
Unknown	2 (20)	3 (7)	9 (13.90
Differentiation, n (%)			
Moderate	8 (80)	17 (39.5)	32 (49.2)
Poor	2 (20)	26 (60.5)	33 (50.8)

replicable. One relatively novel target of investigation in this area is micro-RNAs (miRNAs). These short, noncoding RNA strands function as negative regulators of gene expression.[10] One miRNA can regulate hundreds of targets, leading to a broad variety of effects on cellular function. miRNA expression has been evaluated in the context of sensitivity to therapy in esophageal cancer, with expression of several miRNAs linked to radiotherapy and chemotherapy sensitivity in vitro and in vivo.[11,12] In addition, several small studies have linked expression of individual miRNAs to outcomes in esophageal adenocarcinoma.[13,14] However, to date no studies have provided a robust, replicable predictive model of response to therapy in this disease.

The current study was performed to generate a model of predicting response to chemoradiation in esophageal adenocarcinoma using miRNA expression, with the goal of assisting in the individualized management of this disease.

MATERIALS AND METHODS

Patient Population

Patients with esophageal adenocarcinoma treated with neoadjuvant chemoradiotherapy followed by surgical resection at The University of Texas MD Anderson Cancer Center (UT MDACC) from 2002 to 2009 were eligible for this study. Treated patients with distant metastasis at the time of diagnosis were excluded. A total of 3 patient cohorts treated in this manner were examined: 1) a discovery cohort (n = 10), 2) a model cohort (n = 43), and 3) a validation cohort (n = 65). Clinical characteristics of each patient cohort are seen in Table 1. Pathologists at UT MDACC scored the tumor resection specimens. Pathologic complete response (pCR) was defined as the complete absence of tumor cells in the resected surgical specimen. The UT MDACC institutional review board approved this study.

miRNA Extraction and Expression

RNA was isolated from pretreatment tumor biopsy specimens as described previously.[15] Briefly, miRNA was extracted from pretreatment biopsy specimens using an mirVana miRNA Extraction Kit (Ambion). A NanoDrop Spectrophotometer (Thermal Scientific) was used to quantify the specimens as well as assess the purity of the RNA. In addition, the spectrum of each specimen was visually analyzed to ensure that all profiled samples had a normal spectral profile. These samples were analyzed for miRNA expression using 3 separate platforms: TaqMan Human MicroRNA Card Set v3.0 (Applied Biosystems), Fluidigm 48.48 Dynamic Array (Fluidigm), and Illumina Human MicroRNA expression beadchip v2.0 (Illumina) per the manufacturers' instructions. Our discovery cohort was assayed using a TaqMan array, the model cohort was assayed using a Fluidigm array, and our validation cohort was examined using an Illumina array. This was done to ensure validity across multiple assay platforms. Each miRNA assay was tested in duplicate, and the mean Ct value was normalized to the averaged expression of spike-in miRNAs cel-39 and cel-54 and then subjected to analysis using the $2^{-\Delta\Delta Ct}$ method.[16] For the Taqman array, miRNAs detected in less than 20% of samples were excluded from analysis.

Statistical Analysis

The primary outcome for this study was the presence of pCR after concurrent chemoradiotherapy and surgical resection. In the discovery cohort, a total of 754 miRNAs were analyzed for association with pCR. The Student *t* test and Wilcoxon rank sum test were used to identify miRNAs differentially expressed between tumors with either a pCR or non-pCR. Of these, the most significant 44 miRNAs were analyzed via a Fluidigm array in the model cohort and used to generate the miRNA expression profile (MEP) score as described below. An Illumina miRNA expression array was used to analyze the expression of miRNA in the validation cohort. Individual miRNAs were dichotomized using the median as a cutoff (Fig. 1). In both the model and validation sets, the MEP score for each patient was generated using a linear combination of the product of reference-normalized expression of each dichotomized miRNA by its logistic regression corresponding coefficient.[17]

For pCR prediction, univariate logistic regression was performed and probability of pCR was then determined based on MEP score and either clinical stage or tumor grade based on the estimates from logistic regression. Receiver operating characteristic (ROC) curves and the

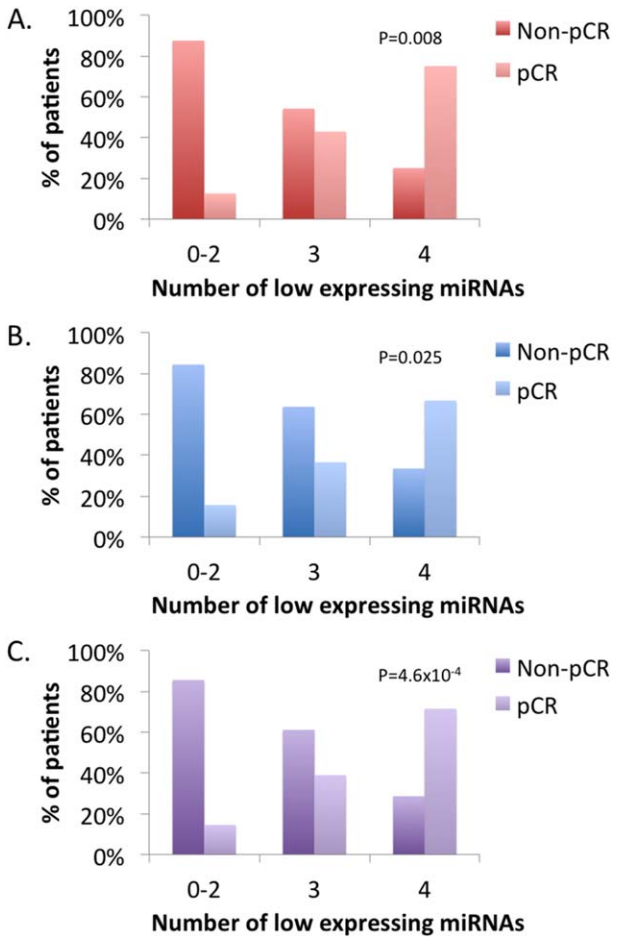


Figure 1. Pathologic complete response (pCR) rates are higher in patients with low levels of selected miRNAa. pCR rates in patients with 0-2, 3, or 4 tumor miRNAs expressed below threshold values in A. the model set, the validation set (B), and the combined model and validation set (C). *P* values represent a highly significant trend of increased pCR rates with increasing numbers of low-expressing miRNA.

corresponding area under the curve (AUC), 95% confidence intervals, and *P* values were generated for the MEP score, clinical stage, tumor grade, and a combination of these variables. Bootstrap resampling was carried out 10,000 times to assess the differences between the AUCs for each of the models. Statistical analyses were done using STATA software (v10, STATA Corporation). All *P* values were 2-sided; with a $P \leq .05$ was considered statistically significant.

RESULTS

Clinical Characteristics

Three separate patient cohorts were used for this analysis. The clinical characteristics of each cohort are shown in Table 1. Disease was staged by using a combination of either computed tomography (CT) or positron emission tomography (PET)/CT and esophagogastroduodenoscopy/endoscopic ultrasonography (EGD/EUS) and with the AJCC 6th edition staging system. All patients were treated with concurrent chemoradiation to a dose of 50.4 Gy, with most receiving 5-fluorouracil (93.4%) and a platinum compound — cisplatin or oxaliplatin) (57%). All patients were then treated with a surgical resection, typically an Ivor-Lewis esophagectomy. Most patients were male (95%) and were relatively evenly balanced in clinical stage (40% II vs 53.4% III or IV) and tumor differentiation (well or moderate 48% vs poor 52%).

miRNA Expression

In the initial discovery cohort a total of 10 pretreatment esophageal tumor samples (5 pCR and 5 non-pCR) were analyzed. A total of 754 miRNAs were evaluated, of which 306 miRNAs showed moderate to high expression in the tumor samples. Median expression of each miRNA for pCR and non-pCR was calculated, and differences between groups were determined by *t* test (Supplemental Table 1). Expression of the miRNAs most able to discriminate between pCR and non-pCR in the discovery cohort were examined in the model cohort with a different platform (Fluidigm). A total of 44 miRNAs was assayed in pretreatment esophageal adenocarcinoma samples from the 43 patients in the model cohort, and differences in median miRNA expression between pCR and non-pCR groups were again determined (Supplemental Table 2). Four miRNAs were significantly differentially expressed between pCR and non-pCR groups in both the discovery and model cohort (miR-505*, miR-99b, miR-451, and miR-145*; Table 2).

miRNA Expression Profile (MEP) Score

To investigate the ability of these 4 miRNAs (termed hereafter as the MEP) to predict pCR, tumor samples were divided into high and low expression of each miRNA. The total number of low-expressing miRNAs for each tumor in the model cohort was determined. As shown in Figure 1A, the number of low-expressing miRNAs was highly associated with the probability of pCR in the model cohort ($P_{trend} = .008$), with tumor samples exhibiting low expression of all 4 miRNAs having a close to 80% pCR rate (Fig. 1A). To validate the ability of the MEP to predict pCR after chemoradiation, we examined the expression of each miRNA in pretreatment tumor samples from a cohort of 65 similarly treated patients using a different platform (validation cohort). Similar to the model cohort, the number of low-expressing miRNAs

TABLE 2. MiRNA Expression in Pretreatment Esophageal Tumors

	Discovery Set			Model Set		
	Non-pCR, median (range)	pCR, median (range)	*P*	Non-pCR, median (range)	pCR, median (range)	*P*
mir-505*	2.16 (1.66-4.46)	1.27 (0.69-1.84)	.05	0.86 (0.68-1.69)	0.54 (0.35-0.78)	.0013
mir-99b	1.94 (1.40-2.92)	1.16 (0.73-1.52)	.016	1.06 (0.83-1.53)	0.73 (0.62-1.23)	.023
mir-451	0.73 (0.49-4.93)	0.27 (0.17-0.40)	.009	0.89 (0.45-2.30)	0.33 (0.15-1.88)	.045
mir-145*	3.02 (1.74-4.91)	1.18 (0.90-1.29)	.009	1.22 (0.68-1.61)	0.76 (0.44-1.36)	.055

Median expression of selected miRNAs from the discovery set (n = 10) assayed via Taqman microRNA assay and the model set (n = 43) assayed by a 48.48 Fluidigm microfluidic dynamic array.
Abbreviation: pCR, pathologic complete response.

in the validation cohort was highly associated with pCR ($P_{trend} = .025$; Fig. 1B). In addition, the number of low-expressing miRNAs was associated with pCR in the combined 2 cohorts ($P_{trend} = 6 \times 10^{-4}$; Fig. 1C).

With these data we created a formula to calculate a risk score for each patient based on the 4 miRNAs in the MEP, weighted by logistic regression coefficient (MEP score). The ability to predict pCR was examined via ROC analysis of sensitivity and specificity for MEP score, clinical stage, tumor grade, and a combination of all 3 (Fig. 2). The MEP score alone was significantly better at predicting pCR compared with common clinical variables in both patient cohorts as well as in the combined data set (Fig. 2). In addition, a combination of clinical factors (stage and grade) with the MEP score significantly improved the model ($P = 3.6 \times 10^{-30}$; Fig. 2).

To provide a clinically useable estimate of the probability of pCR for any given MEP score, estimates from logistic regression were used based on the MEP score and either clinical stage or tumor grade to generate a probability of pCR curve (Fig. 3). As the MEP score increased, the probability of pCR increased in a near-linear fashion, regardless of clinical stage or tumor grade.

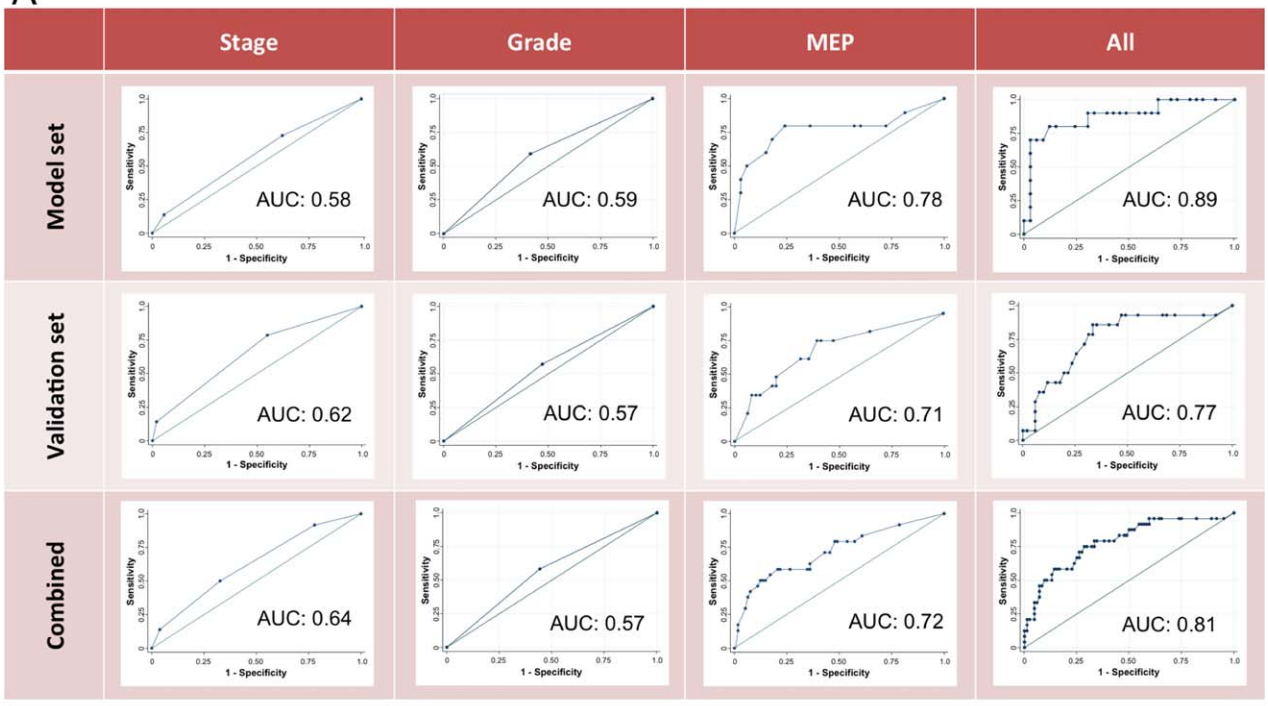
DISCUSSION

In the current study we have demonstrated the first validated miRNA signature to predict response to therapy in esophageal adenocarcinoma. Specifically, an MEP score derived from the expression of 4 miRNAs was highly predictive of pCR after neoadjuvant chemoradiation, across several different assay platforms. In addition, the MEP score could be used in combination with clinical stage and tumor grade to further improve its predictive ability. In this context, a group of patients with a high (>80%) probability of pCR after treatment can be identified.

Several clinical trials have shown the benefit of treating locally advanced esophageal adenocarcinoma with neoadjuvant chemoradiation.[2,18] In fact, some clinicians have advocated reserving surgical resection for patients who present with clinically persistent disease or who present with clinically evident recurrence after chemoradiation. However, there is substantial discordance between clinical and pathologic complete response, with surgical salvage eventually required for a large proportion of patients treated with chemoradiation alone.[5] In addition, disease in some patients will progress during chemoradiation, delaying surgery or even becoming inoperable because of this delay.[9] No method has been found to identify at diagnosis those patients who will likely benefit from neoadjuvant chemoradiation and those patients better suited to upfront surgical resection and possible protocol-based therapy. Thus, a priori knowledge of complete response to therapy is crucial in moving forward with individualized management of this disease.

Much of the previous work examining the clinical relevance of tumor miRNA-based markers has investigated the prognostic significance of a specific miRNA in relatively small groups of patients.[19-27] These data, although interesting, provide no clinically actionable information regarding the management of esophageal cancer. The vast majority of esophageal cancer in the Western world is adenocarcinoma, in contrast with the East, where the predominant histology is squamous cell carcinoma. However, the vast majority of previous studies have either focused on the squamous population or consisted of a mixture of the 2 histologies. Those few studies that have broadly examined tumor miRNA expression to determine response to therapy are no exception. For example, Ko and colleagues examined miRNA expression in 25 esophageal cancer specimens, consisting of a mixture of squamous and adenocarcinoma histologies treated with chemoradiation.[13] In their study, several miRNAs were expressed at significantly different

A



B

	AUC (95% CI)	P value
Model set		
MEP	0.78 (0.52-0.98)	ref
Stage	0.58 (0.51-0.73)	5.42E-24
Grade	0.59 (0.51-0.7)	3.23E-24
All	0.89 (0.72-1)	2.70E-22
Validation set		
MEP	0.71 (0.51-0.84)	ref
Stage	0.62 (0.50-0.78)	1.42E-07
Grade	0.57 (0.51-0.69)	8.36E-21
All	0.77 (0.61-0.94)	4.19E-19
Combined		
MEP	0.72 (0.59-0.83)	ref
Stage	0.64 (0.55-0.74)	2.85E-16
Grade	0.57 (0.50-0.66)	1.27E-34
All	0.81 (0.69-0.91)	3.62E-30

Figure 2. Sensitivity and specificity of the miRNA Expression Profile (MEP) in predicting pathologic complete response (pCR). Receiver operating characteristics (ROC) curves for clinical characteristics of stage and tumor grade, MEP, and a combination of all 3 variables in the model set (n = 43), validation set (n = 65), and both sets of patients combined (n = 114) are shown. *P* values represent comparisons as shown.

levels in pCR and non-pCR; however a profile predicting response could not be generated. Thus, the MEP score represents the first validated, predictive miRNA signature in esophageal adenocarcinoma. In additional, the MEP score was found to be predictive across 3 separate assay platforms, arguing for its broad applicability and against experimental artifact.

The current study did have several weaknesses. Our initial screen of 754 miRNAs did not assess the complete miRNA complement of the tumor; thus, the possibility exists that unprofiled miRNAs may have a greater predictive utility than those examined. In addition, by design, we focused solely on adenocarcinoma histology to provide the most relevant signature for the vast majority of cases of

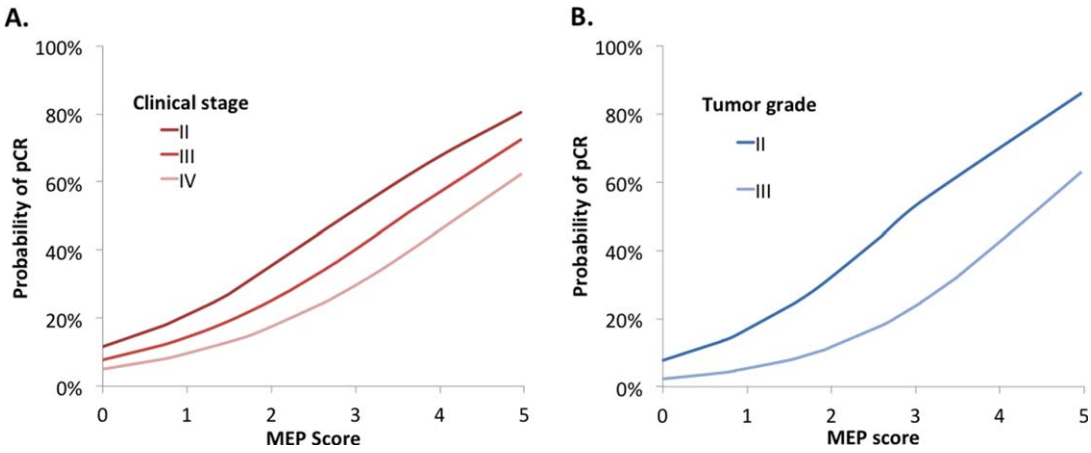


Figure 3. Probability of pathologic complete response (pCR) based on MEP and clinical characteristics. Probability of pCR increases with increasing MEP score as a function of either clinical stage (A.) or tumor grade (B.).

esophageal cancer in the West. Further work profiling miRNA expression solely in squamous cell carcinoma is ongoing.

However, despite these weaknesses, the current study provides a robust platform to identify those patients who are highly favorable candidates for management with chemoradiotherapy alone as well as those patients for whom surgery is necessary and for whom alternate therapies may be appropriate. This is a necessary step in individualizing treatment of esophageal adenocarcinoma.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61:69-90.
2. Van Hagen P, Hulshof MCCM, van Lanschot JJB, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med*. 2012;366(22):2074-2084.
3. Herskovic A, Russell W, Liptay M, Fidler MJ, Al-Sarraf M. Esophageal carcinoma advances in treatment results for locally advanced disease: review. *Ann Oncol*. 2012;23:1095-1103.
4. Crosby T, Hurt CN, Falk S, et al. Chemoradiotherapy with or without cetuximab in patients with oesophageal cancer (SCOPE1): a multicentre, phase 2/3 randomised trial. *Lancet Oncol*. 2013;14:627-637.
5. Swisher SG, Winter KA, Komaki RU, et al. A Phase II study of a paclitaxel-based chemoradiation regimen with selective surgical salvage for resectable locoregionally advanced esophageal cancer: initial reporting of RTOG 0246. *Int J Radiat Oncol Biol Phys*. 2012;82:1967-1972.
6. Sarkaria IS, Rizk NP, Bains MS, et al. Post-treatment endoscopic biopsy is a poor-predictor of pathologic response in patients undergoing chemoradiation therapy for esophageal cancer. *Ann Surg*. 2009;249:764-767.
7. Vallbohmer D, Holscher AH, Dietlein M, et al. [18F] fluorodeoxyglucose-positron emission tomography for the assessment of histologic response and prognosis after completion of neoadjuvant chemoradiation in esophageal cancer. *Ann Surg*. 2009;250:888-894.
8. Erasmus JJ, Munden RF, Truong MT, et al. Preoperative chemoradiation-induced ulceration in patients with esophageal cancer: a confounding factor in tumor response assessment in integrated computed tomographic-positron emission tomographic imaging. *J Thorac Oncol*. 2006;1:478-486.
9. Ajani JA, Wang X, Hayashi Y, et al. Validated biomarker signatures that predict pathologic response to preoperative chemoradiation therapy (CTRT) with high specificity and desirable sensitivity levels in patients with esophageal cancer (EC). *J Clin Oncol*. 2011;29(15 Suppl).
10. Song S, Ajani JA. The role of microRNAs in cancers of the upper gastrointestinal tract. *Nat Rev Gastroenterol Hepatol*. 2013;10:109-118.
11. Mayne GC, Hussey DJ, Watson DI. MicroRNAs and esophageal cancer–implications for pathogenesis and therapy. *Curr Pharm Des*. 2013;19:1211-1226.
12. Hong L, Han Y, Lu Q, et al. Drug resistance-related microRNAs in esophageal cancer. *Expert Opin Biol Ther*. 2012;12:1487-1494.
13. Ko MA, Zehong G, Virtanen C, et al. MicroRNA expression profiling of esophageal cancer before and after induction chemoradiotherapy. *Ann Thorac Surg*. 2012;94:1094-1103.
14. Feber A, Xi L, Pennathur A, et al. MicroRNA prognostic signature for nodal metastases and survival in esophageal adenocarcinoma. *Ann Thorac Surg*. 2011;91:1523-1530.
15. Wu X, Ajani JA, Gu J, et al. MicroRNA expression signatures during malignant progression from Barrett's esophagus to esophageal adenocarcinoma. *Cancer Prev Res*. 2013;6:196-205.
16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25:402-408.
17. Liu H, Zhu L, Liu B, et al. Genome-wide microRNA profiles identify miR-378 as a serum biomarker for early detection of gastric cancer. *Cancer Lett*. 2012;316:196-203.
18. Stahl M, Walz MK, Stuschke M, et al. Phase III comparison of preoperative chemotherapy compared with chemoradiotherapy in patients with locally advanced adenocarcinoma of the esophagogastric junction. *J Clin Oncol*. 2009;27:851-856.
19. Zang W, Wang Y, Du Y, et al. Differential expression profiling of microRNAs and their potential involvement in esophageal squamous cell carcinoma. *Tumour Biol*. 2014;35:3295-3304.

20. Nouraee N, Van Roosbroeck K, Vasei M, et al. Expression, tissue distribution and function of miR-21 in esophageal squamous cell carcinoma. *PLoS One*. 2013;8:e73009.
21. Fu HL, Wu DP, Wang XF, et al. Altered miRNA expression is associated with differentiation, invasion, and metastasis of esophageal squamous cell carcinoma (ESCC) in patients from Huaian, China. *Cell Biochem Biophys*. 2013;67:657-668.
22. Chu Y, Zhu H, Lv L, Zhou Y, Huo J. MiRNA s in oesophageal squamous cancer. *Neth J Med*. 2013;71:69-75.
23. Mathé EA, Nguyen GH, Bowman ED, et al. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. *Clin Cancer Res*. 2009;15:6192-6200.
24. Zhao Y, Schetter AJ, Yang GB, et al. microRNA and inflammatory gene expression as prognostic marker for overall survival in esophageal squamous cell carcinoma. *Int J Cancer*. 2013;132:2901-2909.
25. Hummel R, Hussey DJ, Michael MZ, et al. MiRNAs and their association with locoregional staging and survival following surgery for esophageal carcinoma. *Ann Surg Oncol*. 2011;18:253-260.
26. Hong L, Han Y, Zhang H, et al. The prognostic and chemotherapeutic value of miR-296 in esophageal squamous cell carcinoma. *Ann Surg*. 2010;251:1056-1063.
27. Guo Y, Chen Z, Zhang L, et al. Distinctive microRNA profiles relating to patient survival in esophageal squamous cell carcinoma. *Cancer Res*. 2008;68:26-33.