Key paper evaluation

For Expert Review of Anticancer Therapy
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Abstract/Summary:


Most patients undergoing surgery for esophageal cancer are treated before surgery with chemotherapy and radiotherapy. However, some tumors respond poorly to these treatments. Ko and colleagues profiled microRNA levels in esophageal cancers from patients who did vs. did not respond to chemoradiotherapy. A large number of microRNAs were differentially expressed between responders vs. non-responders, and patients with either decreased miR-135b or increased miR-145 expression in cancer tissue had improved disease free survival. Although this study has several limitations, including a mixed cohort of patients with adenocarcinoma and squamous cell carcinoma, and the absence of a validation set of patients, the results do suggest that a microRNA profiling approach may be able to circumvent one of the primary challenges for biomarker development, molecular heterogeneity.

Word count = 124

Title: concise, not more than 120 characters.

Can microRNA profiling allow us to determine which patients with esophageal cancer will respond to chemoradiotherapy?

Keywords: approximately 5–10 keywords for the review.
microRNA
chemoradiotherapy
esophageal cancer
Body of the article:

Esophageal cancer is the sixth commonest cause of cancer related death in the Western World, and the incidence of esophageal adenocarcinoma (EAC) has increased 6-fold over the past 3 decades in the Western World, especially in men, whilst the incidence of squamous cell carcinoma (SCC) has essentially remained unchanged. The prognosis for both EAC and SCC is poor, with most patients presenting with advanced disease. Hence, much research has centered on the development of pre-surgical (neoadjuvant) chemo- or chemoradiotherapy regimes, and recent meta-analyses suggest that these approaches improve overall survival following surgery for patients with both types of esophageal cancer. However, the clinical response to these treatments is variable, with a significant proportion of patients responding poorly to chemo- or chemoradiotherapy regimes, either in the neoadjuvant, palliative or definitive care settings. Hence, those patients who undergo chemo- or chemoradiotherapy, but respond poorly, don’t benefit, and may even be harmed by treatment. A biological marker, or more likely a panel of markers, that can predict tumor response to chemo- or chemoradiotherapy regimes, might be used to tailor treatment to patients for whom chemo- or chemoradiotherapy is likely to be beneficial, and to avoid it in those unlikely to benefit. The paper published by Ko et al attempts to address this issue, by exploring microRNA expression biomarkers as determinants of response.

MicroRNAs inhibit the expression of target genes at the post-transcriptional level, and are promising candidates as therapy response biomarkers. Recent studies have suggested direct links between miRNAs and the processes leading to cancer, and miRNAs also have a role in tumor biology. There is some evidence that their expression correlates with treatment outcomes. For example, miR-21 expression correlates with the therapeutic outcome in colonic adenocarcinoma [1]. Importantly, there is also evidence that modulation of miRNAs can alter tumor cell sensitivity to chemotherapy in vitro [2].
Methods and results
Ko et al’s study included 25 patients with clinical stage III adenocarcinoma or squamous cell carcinoma of the esophagus who underwent neoadjuvant chemoradiotherapy (irinotecan, cisplatin and radiotherapy ) followed by esophagectomy. Pathology specimens were reviewed by a single pathologist, and patients were separated according to response to therapy. A pathological complete response (pCR) was defined as no viable tumor cells remaining in the surgical specimen. 32% of the 25 patients had a pCR, 60% a partial response, and 8% no response.

For miRNA biomarker evaluation archived formalin fixed paraffin embedded material was obtained pre-treatment by endoscopic biopsy, and post-treatment from the resected surgical specimen. RNA was extracted from 10 µm sections, and hybridized to microarrays. After samples were ordered into test categories, the data were filtered to remove microRNAs that were not above the 20th percentile in more than 50% of samples in any single category.

Samples were classified as complete (pCR) vs incomplete (non-pCR) response, and data were analyzed via two-way unsupervised hierarchical clustering. In the pCR vs. non-pCR clustering, for both pre- vs. post-therapy, miRNA profiling with the complete 1,536 gene set was unable to reliably classify pCR vs. non-pCR, although this approach performed well in distinguishing pretreatment from post-treatment specimens, with 26 of 27 (96%) post-treatment tissues correctly grouped. MiRNA expression differences between groups were then subjected to t tests, and supervised clustering analysis was performed using miRNAs with p<0.05. For pre-treatment specimens, using 71 differentially expressed miRNAs, supervised clustering correctly grouped all patients with a pCR, and correctly grouped 13 of the 17 (76%) non-pCR patients. 51 microRNAs were differentially expressed in the post-treatment tissues between pCR vs. non-pCR, and in supervised clustering 11 of 17 of non-pCR patients were correctly grouped.

Kaplan Meier analysis with log rank tests were used for associations between pCR vs. disease free survival, and between specific miRNAs vs. disease free survival. The
association of pCR vs. disease free survival was not significant, reflecting a small number of patients. High miR-145 was associated with increased disease free survival (11.5 vs. 5.1 months), as was decreased miR-135b (11.5 vs. 2.8 months).

**Discussion & significance**

The results of this study suggest that miRNA expression patterns reflect tumour response to chemoradiotherapy, raising the possibility of using these biomarkers to tailor treatment to patients most likely to benefit. However, there are some methodological issues that might limit the reliability of the findings, and further work will be required to take these concepts forward.

It must be noted that a standard statistical approach to adjust the false discovery rate of the array data was not used. A two step filtering approach was used instead. While two stage filtering approaches have been found to improve detection power over the family-wise error rate or false discovery rate approaches, it has been suggested that this approach can lead to type I errors [3].

The results of the supervised clustering analyses suggest that miRNA profiling to generate response signatures has potential for clinical utility in this context, compared with single-biomarker and even multi-biomarker panels which are limited by molecular heterogeneity [4]. However, it is possible that these results over-estimate the clinical applicability of the profiles, and it is essential that these results are validated in an independent set of tissues that were not used to determine the differentially expressed microRNAs. Also, microarrays are relatively expensive compared with single biomarker assays and the data analysis is not trivial, and for this reason more work needs to be done to demonstrate cost-effectiveness.

The mixed cohort of patients with adenocarcinoma and squamous cell carcinoma adds further complexity. These are histologically different cancers with functionally different levels of miRNA expression. For example, Hu et al (2011) reported that miR-16-2, miR-30e and miR-200a expression were associated with shorter overall and disease-free
survival in patients with esophageal adenocarcinoma, whereas they did not observe an association in esophageal squamous cell carcinomas [5], and Hummel et al (2011) reported that expression of miR-21 correlated with lymph node status in esophageal squamous cell carcinoma but not in adenocarcinoma [6].

However, whilst encouraging, the results must be interpreted within the limitations of the study. The investigators provide no detail about how they determined expression level thresholds for survival analyses, and in future this should be determined via e.g. ROC curve analysis of data from a validation set, with the expression levels of specific miRNAs measured by an alternative method such as real time PCR. The investigators also noted that the results for miR-145 were the opposite of what would be expected given this miRNA’s documented role as a tumor suppressor. This incongruent observation might be the result of increased stromal involvement in tumor formation (e.g. myofibroblasts express high levels of miR-145) [8], and/or the result of transition of epithelial cells to mesenchymal cells in the tumors [9].

**Expert commentary and Five-year view**

The current study suggests that individual miRNAs may be able to identify patients who will have longer disease free survival after neoadjuvant chemoradiotherapy followed by surgery. Arguably more importantly, this study also provides preliminary evidence suggesting that profiling of primary tumors with miRNAs microarrays might differentiate patients with a complete pathological response to pre-operative chemotherapy from non-responders. This type of signature profiling approach may have increased clinical utility in this context compared with single-biomarkers and even multi-biomarker panels which are limited by molecular heterogeneity. Although the mixed cohort of esophageal adenocarcinoma and squamous cell carcinoma patients would be expected to potentially confound the results, supervised hierarchical clustering of 71 microRNAs in pre-therapy biopsies correctly identified all of the patients with a complete pathological response to
chemotherapy in the training set. It is therefore important that these results are validated in a separate cohort of patients, and further validated with another technology such as high throughput real time PCR or deep sequencing.

The investigation of molecular biomarkers for esophageal cancer diagnosis, risk stratification, and prognosis has had limited success, and there is accumulating evidence that this may in part be due to the heterogeneous nature of these diseases. For example, Owonikoko et al (2002) reported inter- and intra-tumoral genetic heterogeneity of various gene amplifications and loss of heterozygosity loci in esophageal adenocarcinoma [10], and Merlo et al (2010) observed that increased diversity of different molecular clones within Barrett’s esophagus was associated with a high risk of progression to esophageal adenocarcinoma [11]. These observations suggest that we may have to reconsider the reductionist approach to biomarker screening and development, and further investigate the possibility that profiling with relatively large numbers of biomarkers may provide greater levels of sensitivity and specificity. A similar conclusion was reached by Kihara et al (2001) based on cDNA microarray investigations of the outcomes of patients with late stage esophageal squamous cancer after chemotherapy: “The usefulness of the prediction of the outcome of adjuvant chemotherapy…raises a possibility that extended analyses of expression profiles with an increased number of genes using a larger number of samples will help in the development of a more accurate classification system.” [12].

**Word Count** 1468

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References


