EXPERT REVIEWS

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

Expert Rev. Anticancer Ther. 13(3), 271–273 (2013)

George C Mayne, Damian J Hussey and David I Watson*

Department of Surgery, Flinders University, Bedford Park, SA 5042, Australia
**Author for correspondence:*
Tel.: +61 882 046 086
Fax: +61 882 046 130
david.watson@flinders.edu.au

Evaluation of: Ko MA, Zehong G, Virtanen C *et al.* miRNA expression profiling of esophageal cancer before and after induction chemoradiotherapy. *Ann. Thorac. Surg.* 94(4), 1094–1103 (2012).

Most patients undergoing surgery for esophageal cancer are treated before surgery with chemotherapy and radiotherapy. However, some tumors respond poorly to these treatments. The article under evaluation profiled miRNA levels in esophageal cancers from patients who did respond to chemoradiotherapy versus those who did not. A large number of miRNAs were differentially expressed between responders versus nonresponders, 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 miRNA profiling approach may be able to circumvent one of the primary challenges for biomarker development, molecular heterogeneity.

Keywords: chemoradiotherapy • esophageal cancer • microRNA

Esophageal cancer is the sixth most common cause of cancer-related death in the Western world. The incidence of esophageal adenocarcinoma (EAC) has increased sixfold over the past three decades in the Western world, especially in men, while 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 presurgical (neoadjuvant) chemotherapy or chemoradiotherapy regimens, 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 chemotherapy or chemoradiotherapy regimens, either in the neoadjuvant, palliative or definitive care settings. Hence, those patients who undergo chemotherapy or chemoradiotherapy, but respond poorly, do not benefit and may even be harmed by treatment. A biological marker, or more likely a panel of markers, that can predict tumor response to chemotherapy or chemoradiotherapy regimens, might be used to tailor treatment to patients for whom chemotherapy 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 miRNA expression biomarkers as determinants of response [1].

miRNAs 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

 10.1586/ERA.12.182 ISSN 1473-7140

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 EAC [2]. Importantly, there is also evidence that modulation of miRNAs can alter tumor cell sensitivity to chemotherapy *in vitro* [3].

Methods & results

Ko *et al.*'s study included 25 patients with clinical stage III EAC or SCC 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. Thirty two percent of the 25 patients had pCR, 60% had partial response and 8% had no response.

For miRNA biomarker evaluation, archived formalin-fixed paraffin-embedded material was obtained pretreatment 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 miRNAs that were not above the 20th percentile in more than 50% of samples in any single category.

Samples were classified as complete (pCR) versus incomplete (non-pCR) response, and data were analyzed via two-way unsupervised hierarchical clustering. In the pCR versus non-pCR clustering, for both pre- versus post-therapy, miRNA profiling with the complete 1536-gene set was unable to reliably classify pCR versus non-pCR, although this approach performed well in distinguishing pretreatment from post-treatment specimens, with 26 out of 27 (96%) post-treatment tissues being 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 pretreatment specimens, using 71 differentially expressed miRNAs, supervised clustering correctly grouped all patients with a pCR, and correctly grouped 13 out of the 17 (76%) non-pCR patients. Fifty one miRNAs were differentially expressed in the post-treatment tissues between pCR versus non-pCR, and in supervised clustering, 11 out of 17 of non-pCR patients were correctly grouped.

Kaplan–Meier analysis with log-rank tests were used for associations between pCR versus disease-free survival and between specific miRNAs versus disease-free survival. The association of pCR versus 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 tumor 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 may 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 [4].

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 [5]. However, it is possible that these results overestimate 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 miRNAs. 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 EAC and SCC adds further complexity. These are histologically different cancers with functionally different levels of miRNA expression. For example, Hu *et al.* reported that miR-16-2, miR-30e and miR-200a expression were associated with shorter overall and disease-free survival in patients with EAC, whereas they did not observe an association in esophageal SCCs [6], and Hummel *et al.* reported that expression of miR-21 correlated with lymph node status in esophageal SCC but not in EAC [7].

However, while 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, for example, receiver operating characteristic 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 may 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 the transition of epithelial cells to mesenchymal cells in the tumors [9].

Expert commentary & 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 miRNA microarrays may differentiate patients with a complete pathological response to preoperative chemotherapy from non-responders. This type of signature profiling approach may have increased clinical utility in this context compared with single biomarker and even multi-biomarker panels which are limited by molecular heterogeneity. Although the mixed cohort of EAC and SCC patients would be expected to potentially confound

the results, supervised hierarchical clustering of 71 miRNAs in pretherapy 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*. reported inter- and intra-tumoral genetic heterogeneity of various gene amplifications and loss of heterozygosity loci in EAC [10], and Merlo *et al*. observed that increased diversity of different molecular clones within Barrett's esophagus was associated with a high risk of progression to EAC [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*. 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].

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1 Ko MA, Zehong G, Virtanen C *et al*. miRNA expression profiling of esophageal cancer before and after induction chemoradiotherapy. *Ann. Thorac. Surg.* 94(4), 1094–1103 (2012).

2 Schetter AJ, Leung SY, Sohn JJ *et al*. miRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA* 299(4), 425–436 (2008).

3 Hummel R, Watson DI, Smith C *et al*. Mir-148a improves response to chemotherapy in sensitive and resistant oesophageal adenocarcinoma and squamous cell carcinoma cells. *J. Gastrointest. Surg.* 15(3), 429–438 (2011).

4 Bourgon R, Gentleman R, Huber W. Independent filtering increases detection power for high-throughput experiments. *Proc. Natl Acad. Sci. USA* 107(21), 9546–9551 (2010).

5 Bhatia S, Frangioni JV, Hoffman RM, Iafrate AJ, Polyak K. The challenges posed by cancer heterogeneity. *Nat. Biotechnol.* 30(7), 604–610 (2012).

6 Hu Y, Correa AM, Hoque A *et al*. Prognostic significance of differentially expressed miRNAs in esophageal cancer. *Int. J. Cancer* 128(1), 132–143 (2011).

7 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.* 18(1), 253–260 (2011).

8 Cordes KR, Sheehy NT, White MP *et al*. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* 460(7256), 705–710 (2009).

9 López-Lago MA, Thodima VJ, Guttapalli A *et al*. Genomic deregulation during metastasis of renal cell carcinoma implements a myofibroblast-like program of gene expression. *Cancer Res.* 70(23), 9682–9692 (2010).

10 Owonikoko T, Rees M, Gabbert HE, Sarbia M. Intratumoral genetic heterogeneity in Barrett adenocarcinoma. *Am. J. Clin. Pathol.* 117(4), 558–566 (2002).

11 Merlo LM, Shah NA, Li X *et al*. A comprehensive survey of clonal diversity measures in Barrett's esophagus as biomarkers of progression to esophageal adenocarcinoma. *Cancer Prev. Res. (Phila.)* 3(11), 1388–1397 (2010).

12 Kihara C, Tsunoda T, Tanaka T *et al*. Prediction of sensitivity of esophageal tumors to adjuvant chemotherapy by cDNA microarray analysis of gene-expression profiles. *Cancer Res.* 61(17), 6474–6479 (2001).