



# miRNA profile as a predictor of response to chemoradiation therapy for esophageal squamous cell carcinoma

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## Introduction

Esophageal cancer is estimated to be the eighth most common cancer in the world (456,000 cases) and the sixth most common cause of death (400,000 deaths) (1). Esophageal squamous cell carcinoma (ESCC) is one of the major histological types of esophageal cancer. Despite the development of multimodality therapies such as surgery, chemotherapy, and radiation therapy, the prognosis of ESCC patients remains dismal. Given this background, predicting the clinical and prognostic outcome of ESCC patients is of considerable importance.

Micro-RNAs (miRNAs) are non-coding RNAs containing approximately 22 nucleotides that post-transcriptionally regulate gene expression by base pairing to partially complementary sequences in the 3'-Untranslated region of their target messenger RNA (mRNA). In this way, cell phenotypes such as proliferation, apoptosis, and differentiation are regulated during mammalian development by miRNAs (2). Alterations in miRNA expression affect gene expression and signaling pathways and can therefore lead to several types of diseases, including neurological, cardiovascular, infectious, and inflammatory disorders. Regarding cancer, altered miRNA expression can result in changes in the expression of both oncogenes and tumor suppressors, which in turn regulate the proliferation, apoptosis, motility, and invasiveness of gastrointestinal cancer cells, including those of ESCC (3). Therefore, altered miRNA expression profiles might have clinical potential as useful biomarkers (3). In several cancers,

miRNA expression has been used to detect response to chemoradiation, suggesting that miRNA profiling might lead to personalized therapy (4).

Jing Wen and colleagues recently published a report in which they assessed the efficacy of pretreatment miRNA profiles to predict pathological response to preoperative chemoradiation (5). This study consisted of two phases: 27 ESCCs in the training set and 79 ESCCs in the validation set. Patients with pathological complete response (0% residual cancer in the surgical specimen) or partial response (1% to 50% residual cancer) were defined as responders to preoperative chemoradiation. In the training set, 22 pathological responders and 5 non-responders were compared using Agilent human miRNA microarrays based on miRBase and 26 miRNAs were found to be differentially expressed between these two groups with greater than 1.5-fold change. Among these miRNAs, 10 exhibited a greater than 1.5-fold change between pathological responders and non-responders in verification by real-time quantitative polymerase chain reaction (qPCR) with the same samples as used for microarray analysis. These 10 miRNAs were further validated by qPCR in 79 FFPE samples of the validation set. Next, a combination of miR-145-5p, miR-152, miR-193b-3p, and miR-376a-3p was shown to be a predictive marker for response with overall predictive accuracy of 87.3% (69/79), sensitivity of 83.3% (10/12), and specificity of 88.1% (59/67). These findings indicate that miRNA expression profiling has potential as a biomarker that might lead to individualized therapy for patients with ESCC. However, larger number of patients should be studied and prospective validation would

be important.

### Preoperative chemoradiotherapy for ESCC

Preoperative therapy is standard of care for operable ESCC (6). A large multicenter study from the Netherlands (CROSS trial) revealed that preoperative chemoradiation followed by surgery significantly improved prognosis compared with surgery alone (7). The median overall survival was 48.6 months in the chemoradiation/surgery group and 24.0 months in the surgery alone group (8). Incidentally, in the CROSS trial, 49% of ESCC patients achieved a pathologically complete response (pCR). In contrast, the pCR rate of esophageal adenocarcinoma (EAC) was 23%, suggesting that ESCC is more sensitive to chemoradiation than EAC (7). It remains unclear whether patients who would achieve a pCR from preoperative chemoradiation need to undergo esophagectomy. Two randomized studies have evaluated the efficacy of surgery after induction chemotherapy for resectable ESCC. The FFCO 9102 study randomly allocated patients who were responders to chemoradiation (two cycles of fluorouracil and cisplatin, 30–45 Gy) into a surgery group and continuation of chemoradiation, but reported no benefit for the addition of surgery after chemoradiation compared with the continuation of additional chemoradiation (9), however, this study has considerable shortcomings. In another study from Germany in which patients were randomly assigned to receive induction chemotherapy followed by chemoradiation (40 Gy) followed by surgery, or followed by chemoradiation (65 Gy) without surgery. The addition of surgery after chemoradiation improved local tumor control but did not increase overall survival (10). These studies suggest that if we could identify patients who have exquisitely sensitive tumor to chemoradiation, we may be able to avoid surgery in them. Conversely, non-responders could avoid chemoradiation, which might be detrimental because of the increased toxicity and delay of curative surgery. The Municon I and II trials evaluated a PET (positron emission tomography)-guided treatment algorithm in esophageal cancer, which used PET as an early chemotherapy response indicator and then, changed the treatment strategy according to PET response (11,12). These trials demonstrated that primary resistance cannot be overcome by simply changing therapy empirically and will require in depth study of the tumors to be able to personalize therapy. If chemoradiation is likely to be ineffective, it should be avoided before surgery. Importantly, esophagectomy is a

surgery with an extremely high postoperative morbidity rate, especially after preoperative therapy (13).

### miRNA profile predicts response to chemoradiation

Identification of the molecular behavior of ESCC might allow us to predict response to chemoradiation. Recently, miRNAs have been shown to play an important role in the regulation of several biological processes, including cell growth, apoptosis, and carcinogenesis, leading to the development of chemosensitivity or chemoresistance in several cancers. In rectal cancer, miR-223 has been found to be a predictor of response to chemoradiation using preoperative biopsy tissue samples (4). Indeed, miRNAs have been implicated in resistance mechanisms against chemotherapy or radiotherapy in ESCC. For example, miR-21, which is a well-known onco-miR, inhibits PTEN and thus increases the resistance of ESCC tumors to radiotherapy (14). Moreover, ESCC patients with good response to chemotherapy using the docetaxel/cisplatin/5-fluorouracil (DCF) regimen have significantly low serum miR-21 concentrations (15). Interestingly, miR-200c, which plays an essential role in regulating the epithelial mesenchymal transition, has been shown to facilitate resistance to preoperative chemotherapy using cisplatin/adriamycin/5-fluorouracil through regulation of the Akt pathway (16). The serum miR-24 level in ESCC patients who are CRT responders differs from that in non-responders, suggesting that miR-24 has the potential to serve as a biomarker for predicting chemoradiation responses (17). miRNA has been shown to stimulate cancer-associated fibroblasts, leading to modulation of treatment response. miR-27a/b cause resistance to cisplatin-based chemotherapy through stimulating the transformation of normal fibroblasts into cancer-associated fibroblasts (18). Thus, several miRNAs are associated with treatment response in ESCC, suggesting that miRNA profiling might play a key role in predicting patients who are chemoradiation responders or non-responders.

### Perspective for personalized therapy for ESCC

Development of molecular biology technologies has led to personalized cancer therapy for ESCC by dividing patients into several subgroups according to gene, miRNA, or protein expression, DNA mutation, and epigenetic changes. Wen *et al.* compared gene expression between 11 pCR and 17 non-pCR ESCC patients after

preoperative chemoradiation using pretreated biopsy samples, and proposed *MMP1*, *LIMCH1*, and *C1orf226* as potential predictive biomarkers for response to preoperative chemoradiation (19). Tong *et al.* focused on long non-coding RNA and proposed that low expression of LOC285194 might be an independent prognostic factor for preoperative chemoradiation response using pretreatment biopsy specimens (20). Using a proteomics approach, Zhao *et al.* showed that pretreatment serum C4a and C3a levels were significantly higher in non-responders than responders for neoadjuvant chemoradiation (21). In addition, methylation of checkpoint with forkhead and ring finger domains (*CHFR*) has been reported to be a sensitive marker for response to docetaxel or paclitaxel chemotherapy (22). Although several approaches have been attempted to identify useful molecular markers that predict chemo- or radiotherapy response, the results have not applied to clinical practice. Therefore, validation in a prospective study with a large-scale population must be a focus of future studies.

## Conclusions

In summary, identifying predictive markers for response to preoperative chemoradiation in ESCC is needed to develop personalized therapy and further study should be continued. Expression levels of miRNAs are potential predictive biomarkers of chemoradiation response.

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## References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359-86.
2. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 2004;5:522-31.
3. Song JH, Meltzer SJ. MicroRNAs in pathogenesis, diagnosis, and treatment of gastroesophageal cancers. *Gastroenterology* 2012;143:35-47.e2.
4. Hotchi M, Shimada M, Kurita N, et al. microRNA expression is able to predict response to chemoradiotherapy in rectal cancer. *Mol Clin Oncol* 2013;1:137-42.
5. Wen J, Luo K, Liu H, et al. MiRNA Expression Analysis of Pretreatment Biopsies Predicts the Pathological Response of Esophageal Squamous Cell Carcinomas to Neoadjuvant Chemoradiotherapy. *Ann Surg* 2016;263:942-8.
6. Baba Y, Watanabe M, Yoshida N, et al. Neoadjuvant treatment for esophageal squamous cell carcinoma. *World J Gastrointest Oncol* 2014;6:121-8.
7. van Hagen P, Hulshof MC, van Lanschot JJ, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med* 2012;366:2074-84.
8. Shapiro J, van Lanschot JJ, Hulshof MC, et al.

- Neoadjuvant chemoradiotherapy plus surgery versus surgery alone for oesophageal or junctional cancer (CROSS): long-term results of a randomised controlled trial. *Lancet Oncol* 2015;16:1090-8.
9. Bedenne L, Michel P, Bouché O, et al. Chemoradiation followed by surgery compared with chemoradiation alone in squamous cancer of the esophagus: FFCD 9102. *J Clin Oncol* 2007;25:1160-8.
  10. Stahl M, Stuschke M, Lehmann N, et al. Chemoradiation with and without surgery in patients with locally advanced squamous cell carcinoma of the esophagus. *J Clin Oncol* 2005;23:2310-7.
  11. zum Büschenfelde CM, Herrmann K, Schuster T, et al. (18)F-FDG PET-guided salvage neoadjuvant radiochemotherapy of adenocarcinoma of the esophagogastric junction: the MUNICON II trial. *J Nucl Med* 2011;52:1189-96.
  12. Lordick F, Ott K, Krause BJ, et al. PET to assess early metabolic response and to guide treatment of adenocarcinoma of the oesophagogastric junction: the MUNICON phase II trial. *Lancet Oncol* 2007;8:797-805.
  13. Klevebro F, Johnsen G, Johnson E, et al. Morbidity and mortality after surgery for cancer of the oesophagus and gastro-oesophageal junction: A randomized clinical trial of neoadjuvant chemotherapy vs. neoadjuvant chemoradiation. *Eur J Surg Oncol* 2015;41:920-6.
  14. Huang S, Li XQ, Chen X, et al. Inhibition of microRNA-21 increases radiosensitivity of esophageal cancer cells through phosphatase and tensin homolog deleted on chromosome 10 activation. *Dis Esophagus* 2013;26:823-31.
  15. Kurashige J, Kamohara H, Watanabe M, et al. Serum microRNA-21 is a novel biomarker in patients with esophageal squamous cell carcinoma. *J Surg Oncol* 2012;106:188-92.
  16. Hamano R, Miyata H, Yamasaki M, et al. Overexpression of miR-200c induces chemoresistance in esophageal cancers mediated through activation of the Akt signaling pathway. *Clin Cancer Res* 2011;17:3029-38.
  17. Dong W, Li B, Wang Z, et al. Clinical significance of microRNA-24 expression in esophageal squamous cell carcinoma. *Neoplasma* 2015;62:250-8.
  18. Tanaka K, Miyata H, Sugimura K, et al. miR-27 is associated with chemoresistance in esophageal cancer through transformation of normal fibroblasts to cancer-associated fibroblasts. *Carcinogenesis* 2015;36:894-903.
  19. Wen J, Yang H, Liu MZ, et al. Gene expression analysis of pretreatment biopsies predicts the pathological response of esophageal squamous cell carcinomas to neo-chemoradiotherapy. *Ann Oncol* 2014;25:1769-74.
  20. Tong YS, Zhou XL, Wang XW, et al. Association of decreased expression of long non-coding RNA LOC285194 with chemoradiotherapy resistance and poor prognosis in esophageal squamous cell carcinoma. *J Transl Med* 2014;12:233.
  21. Zhao J, Fan YX, Yang Y, et al. Identification of potential plasma biomarkers for esophageal squamous cell carcinoma by a proteomic method. *Int J Clin Exp Pathol* 2015;8:1535-44.
  22. Yun T, Liu Y, Gao D, et al. Methylation of CHFR sensitizes esophageal squamous cell cancer to docetaxel and paclitaxel. *Genes Cancer* 2015;6:38-48.

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