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REVIEW

Current status of predictive biomarkers for neoadjuvant therapy in esophageal cancer

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Abstract

Neoadjuvant therapy has been proven to be extremely valuable and is widely used for advanced esophageal cancer. However, a significant proportion of treated patients (60%-70%) does not respond well to neoadjuvant treatments and develop severe adverse effects. Therefore, predictive markers for individualization of multimodality treatments are urgently needed in esophageal cancer. Recently, molecular biomarkers that predict the response to neoadjuvant therapy have been explored in multimodal approaches in esophageal cancer and successful examples of biomarker identification have been reported. In this review, promising candidates for predictive molecular biomarkers developed by using multiple molecular approaches are reviewed. Moreover, treatment strategies based on the status of predicted biomarkers are discussed, while considering the international differences in the clinical background. However, in the absence of adequate treatment options related to the results of the biomarker test, the usefulness of these diagnostic tools is limited and new effective therapies for biomarker-identified nonresponders to cancer treatment should be concurrent with the progress of predictive technologies. Further improvement in the prognosis of esophageal cancer patients can be achieved through the introduction of novel therapeutic approaches in clinical practice.

Key words: Esophageal cancer; Neoadjuvant therapy; Response prediction; Molecular biomarker; Chemoradiation

Core tip: To achieve individualization of neoadjuvant therapy for locally advanced esophageal cancers, predictive biomarkers are urgently needed. Biomarker development using multimodal approaches, including gene expression profiling, single nucleotide polymorphisms, microRNAs, proteomics, immunohistochemistry, serum biomarkers and conventional blood tests, seem promising. Independent validation studies will establish novel prognostic modalities based on molecular biomarkers. Progress of predictive modalities and further studies on the molecular background of patients with a poor prognosis will facilitate the development of new effective therapies for patients resistant to the present neoadjuvant therapy. Prognostic stratification of patients will promote efforts toward novel therapeutic strategies.

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INTRODUCTION

Esophageal cancer is the fifth most common cause of cancer-related death for men and the eighth for women worldwide[1]. Despite the use of modern surgical tech-

niques in combination with radio- and chemotherapy, early recurrence is common and the overall 5-year survival rate remains below 40%[2]. Consequently, there is a great interest in multimodal approaches to the treatment of esophageal cancer and neoadjuvant chemotherapy, alone or in combination with chemoradiotherapy (CRT), is becoming the standard approach of care in locally advanced esophageal cancers. Randomized trials of different neoadjuvant therapy protocols have been conducted in patients with locally advanced cancers. Meta-analyses of those randomized trials have revealed only modest survival advantages, except in the case of patients who achieved a complete histopathological response and seemed to highly benefit from a neoadjuvant regimen[3-9]. However, a significant proportion (60%-70%) of treated patients did not respond well to these treatments and experienced severe adverse effects[8,10]. In addition, nonresponsive patients may lose the option of surgical resection after ineffective chemotherapy[11] and the prognosis of nonresponders has been found to be inferior to that for patients treated by surgery alone[12]. While there is an obvious correlation between the response and prognosis, the response to chemotherapy or radiotherapy is variable, even when patients are at the same clinical stage. Thus, an accurate risk stratification of cancer patients for therapy is of paramount importance for avoiding potential morbidity due to ineffective treatment and prevention of further disease progression. With this background, identification of predictive markers would allow accurate risk stratification and individualization of multimodality treatment for patients with locally advanced esophageal cancer[13].

In recent years, molecular biomarkers that can predict the response to neoadjuvant therapy in esophageal cancer have been investigated by using multidimensional approaches. Global expression transcriptomics and proteomics studies allow for simultaneous screening of several thousand molecules and knowledge-based methodologies such as immunohistochemistry are focused on a specific molecule or pathway. These approaches are based on their own unique principles and the performance of predictive molecular biomarkers developed by using each approach seems to be equally promising. Here, we have reviewed the current status of molecular biomarkers predictive for response to neoadjuvant therapy in esophageal cancer. We have focused on predictive markers that can be used to analyze pretreatment samples such as diagnostic biopsies or serum specimens obtained before neoadjuvant treatment. These biomarkers will help avoid unnecessarily invasive treatments. We have summarized promising candidates for predictive molecular biomarkers in esophageal cancer according to the type of development modality.

MOLECULAR BIOMARKERS FOR RESPONSE PREDICTION

Gene expression profiling

High throughput technology such as gene expression microarray has been considered as one of the most powerful tools for understanding the biological characteristics of malignancies. Microarray-based gene expression profiling generates quantitative expression data for thousands of genes, which can be further analyzed by various bioinformatics approaches to identify the most informative genes relevant to cancer prognosis. In particular, the gene expression signatures determined by microarrays have been used to predict the response to neoadjuvant treatment among cancer patients[14].

Maher *et al*[15] investigated gene expression profiles in a cohort comprising 13 patients who were the most responsive or resistant to a standard combination of chemotherapy and radiation therapy. The authors identified five genes (*EPB41L3*, *RNPC1*, *RTKN*, *STAT5B* and *NMES1*) as predictive biomarkers by using DNA microarrays and validated the results by qRT-PCR, confirming that the expression level of five genes could be used to predict the response to neoadjuvant CRT in esophageal cancer with 95% accuracy. Luthra *et al*[16] profiled pretreatment endoscopic cancer biopsies from 19 patients using an AffymetrixU133A Chip (Santa Clara, CA) and noted correlation of the molecular profiles with pathological response to neoadjuvant treatments. The authors reported that the expression levels of three genes (*PERP*, *S100A2 and SPRR3*) helped discriminate between patients with complete histopathological response and those resistant to treatment, with high sensitivity (86%) and specificity (85%). Schauer *et al*[17] performed microarray analysis in 47 patients who had a locally advanced esophageal adenocarcinoma (AC) and had undergone neoadjuvant chemotherapy with cisplatin, leucovorin and 5-fluorouracil, followed by resection. The authors found that the gene encoding the ephrin B3 receptor showed the most prominent differential expression between responders and nonresponders and validated these results by immunohistochemistry. Motoori *et al*[18] performed comprehensive gene expression profiling of pretreatment biopsy samples from 25 patients with esophageal squamous cell carcinoma (SCC) to identify expression patterns predictive for cisplatin-based neoadjuvant chemotherapy. Their system consisted of 199 most informative genes and had the prediction accuracy of 82%. Duong *et al*[19] performed microarray analysis for 46 esophageal cancer patients, that is, 21 SCC and 25 AC patients for whom neoadjuvant CRT had been recommended. Their study was based on two-color competitive hybridization to a cDNA array printed at the Peter MacCallum Cancer Centre Microarray Core Facility[19] and identified a 32-gene classifier that could be used to predict a response to neoadjuvant CRT in SCCs, whereas a negative predictive profile was observed for AC patients.

These examples suggest that gene expression profiling is a powerful tool to identify gene sets for selection of optimal and personalized therapy for patients with esophageal cancer. In breast cancer, mRNA expression signatures strongly predictive of metastasis have been identified and a novel prognostic test for assessing the

risk of metastasis and benefits of chemotherapy has been introduced in clinical settings. This test, named MammaPrint, effectively identifies breast cancer patients with a high risk of recurrence after local treatment alone[20]. The Oncotype DX assay (Genomic Health, Redwood, CA) is another test aimed at better discerning breast cancer patients who would benefit from chemotherapy and those who can safely avoid it. By using the Oncotype DX, we measured the status of 21 genes and could predict the benefits of chemotherapy and the rate of cancer recurrence in 10 years[21]. Similar diagnostic predictive tests are desired for esophageal cancer; however, in this case, different prognostic biomarkers have been identified by using similar technical platforms. The results of these studies need further validation in order to forward their clinical application.

Single nucleotide polymorphisms

In the process of generating a draft sequence of the human genome, it has become clear that the extent of genetic variation is much larger than previously estimated[22,23]. The most common sequence variation in the human genome is the stable substitution of a single base called single-nucleotide polymorphism (SNP). By definition, SNP has a minor allele frequency of greater than 1% in at least one population[24]. Most SNPs are silent and do not alter gene expression or function. The cancer genomics research on SNP variation provides an opportunity for the detection of molecular biomarkers predictive of the response to cancer therapy[25].

Wu *et al*[26] investigated the association between SNPs in multigenic cascades involved in radiation and chemotherapy-dependent responses and clinical outcomes for esophageal cancer patients. The authors applied the pathway-based approach to examine the impact of a comprehensive SNP panel on clinical outcomes in 210 esophageal cancer patients and found that among the genes involved in DNA base excision repair, the variant alleles R399Q in the *XRCC1* gene were significantly associated with the absence of complete pathological response and poor survival. Warnecke-Eberz *et al*[27] investigated a panel of selected gene SNPs to predict responses to neoadjuvant radiochemotherapy in 52 esophageal cancer patients. The authors showed that SNP of C118T in the *ERCC1* gene and the rarely occurring AA genotype of the *XRCC1* gene were predictive of therapy response. Both *ERCC1* and *XRCC1* genes are components of the nucleotide excision repair pathway that protects the integrity of the genome by removing a wide variety of DNA lesions including inter- and intra-strand crosslinks caused by platinum agents or radiation[28]. These SNPs in *ERCC1* appeared to have functional significance because a low intra-tumoral expression of the ERCC1 protein was found to be strongly associated with a major pathological response[29,30]. Moreover, Brabender *et al*[31] reported that *ERCC1* RNA expression in peripheral blood could be a predictor of the response to neoadjuvant therapy. Functional contribution of SNPs in other genes involved in nucleotide excision repair should be investigated for further understanding of the pathogenesis of esophageal cancer.

Clinical applications of SNP testing in cancer are quite realistic. In other types of cancer, the cancer genomics research on SNP variation has provided clinical applications. For example, genetic polymorphisms of the *UGT1A1* gene would affect inter-individual variations in the toxic response to irinotecan by altering the bioavailability of the irinotecan active metabolite SN-38[32,33]. Genetic testing for the presence of the UGT1A1*28 allele has been approved by the FDA and has become available in hospitals. Similar tests for genetic polymorphisms in esophageal cancer would be extremely useful and validation studies for the predictive potential of SNPs would promote their introduction in clinics.

MicroRNAs

MicroRNAs (miRNAs) are short (19-24 nucleotides) noncoding RNA sequences involved in the regulation of gene expression *via* the inhibition of mRNA translation[34,35]. Many lines of evidence suggest that miRNAs exist stably in tissues and body fluids and play a key role in various biological processes, including carcinogenesis. Aberrant miRNA expression has been shown to correlate with the inhibition of tumor suppressor genes or inappropriate activation of oncogenes. Recent studies have shown that the abnormal miRNA expression patterns frequently detected in esophageal cancers have strong prognostic values[36-39]. The predictive utility of miRNAs has also been demonstrated by global expression studies.

Odenthal *et al*[40] assessed miRNA profiles of responders and nonresponders to neoadjuvant therapy for esophageal cancer in order to identify possible predictive markers. The authors found that the pre-therapeutic intra-tumor expression of miR-192 and miR-194 was significantly associated with the histopathological response of esophageal SCCs to multimodal therapy. Using pretreatment biopsy specimens, Ko *et al*[41] showed that the miRNA expression profile was significantly different between groups with and without complete pathological response. Among the 71 differentially regulated miRNAs, five showed the difference of more than two-fold; these included miR-296[42], which has recently been shown to be of prognostic significance in esophageal cancer. The inhibition of miR-296 also resulted in the increased chemosensitivity of esophageal cancer cells to standard chemotherapeutic agents such as 5-fluorouracil and cisplatin[42]. Tanaka *et al*[43] investigated the serum levels of miR-21, miR-145, miR-200c and let-7c by qRT-PCR in 64 esophageal cancer patients treated with neoadjuvant chemotherapy. The authors revealed a significant correlation of miR-200c high expression with poor response to chemotherapy. The possible prognostic utility of miR-200c was also reported by Hamano *et al*[44], who in a study of 98 patients found that miR-200c was involved in resistance to chemotherapy. Lynam-Lennon *et al*[45] demonstrated that resistance to radiation was sig-

nificantly associated with the downregulation of miR-31 and that the ectopic re-expression of miR-31 considerably restored radiosensitivity of the resistant cells. The authors also showed that miR-31 expression was markedly reduced in patients with poor pathological response to neoadjuvant CRT, whereas the expression of the miR-31-regulated DNA repair genes significantly increased[45].

Clinical application of miRNAs as predictive biomarkers is quite feasible because miRNAs are relatively stable and their expression levels can be quantitatively assessed by qRT-PCR. Currently, several clinical trials have already been approved by the FDA to evaluate the value of serum miRNAs in therapeutic response prediction (http://clinicaltrials.gov). Clinical trials evaluating serum miRNAs include the search for predictors of therapeutic response in ovarian carcinoma and miRNA profiling of breast cancer in patients undergoing neoadjuvant or adjuvant treatment[46]. Further functional studies would hopefully validate the functional relevance of miRNAs in esophageal cancer and result in diagnostic and novel therapeutic approaches.

Proteomics

The proteome is a functional translation of the genome. The genomic aberrations in cancer cells are translated to the proteome determining cancer phenotypes and regulating tumor behavior. Because proteins are the main executioner biomolecules, which influence the molecular pathways in normal and tumor cells, proteomic markers are closer and more relevant to cancer initiation and progression than other biomarkers. Proteomic studies can therefore generate unique data related to cancer phenotypes. Many lines of evidence have demonstrated the discordance between mRNA and protein expression[47-49]. In addition, DNA sequence and mRNA expression cannot accurately predict post-translational modifications such as phosphorylation and glycosylation, which play a key role in regulating the malignant behavior of cancer cells. Taken together, proteomic studies can provide valuable information for biomarker identification in various cancers[50-52].

Aichler *et al*[53] analyzed proteomic changes associated with response to chemotherapy by MALDI imaging mass spectrometry using pre-therapeutic biopsy samples of 23 esophageal ACs. Proteins related to clinical response were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The authors discovered that clinical response to cisplatin was associated with the defects in the mitochondrial respiratory chain of cancer cells caused by the loss of specific cytochrome c oxidase subunits. Maher *et al*[54] examined the proteomic profiles of serum samples by using surface-enhanced laser desorption/ionization time-of-flight (SELDL-TOF) mass spectrometry and validated the results with an enzyme-linked immunosorbent assay. By comparing pre-treatment serum samples from 16 poor responders and 15 good responders, the authors found that higher serum levels of complement factors C4a and C3a were significantly associated with favorable response to treatments. The leave-one-out cross-validation analysis revealed that these serum proteins could predict the response to neoadjuvant CRT with a sensitivity and specificity of 78.6% and 83.3%, respectively.

Although there are various reports about biomarker candidates identified by proteomics studies, only a few of them have been proven to be clinically useful[55] because of the lack of independent validation studies. However, the prognostic utility of protein biomarkers has been successfully validated for gastrointestinal stromal tumors in extensive multi-institutional studies[56]. Further validation studies will promote the clinical application of promising protein biomarkers for esophageal cancer.

Immunohistochemistry

By focusing on functionally important molecules or pathways, discovery of biomarker candidates can be performed effectively. Global expression studies based on statistical data may not be able to identify functionally important genes and proteins because expression levels do not always reflect functional activity. In this sense, a knowledge-dependent approach such as immunohistochemistry has unique advantages over the other methods for expression assessment because it allows for the analysis of a large number of formalin-fixed and paraffin-embedded tissue sample archives and provides detailed spacious information not available by other methods. Immunohistochemistry has been successfully used for hypothesis-driven biomarker discovery[57].

Solid tumors are driven and managed by a small population of cancer stem cells (CSCs), tumor-initiating cells or cancer stem-like cells[58-61]. Among these cells, CSCs are found to be more resistant to treatment[62,63]; therefore, CSC markers have been considered promising candidates for predictive biomarkers. Previous reports have demonstrated the importance of CSC markers including growth factor receptors, tumor suppressor genes and DNA-repair pathway factors in malignant features of esophageal cancer cells. Smit *et al*[64] investigated the expression of CSC markers, *in vitro* growth of spheroids, sensitivity to radiation and *in vivo* growth of several esophageal cancer-derived cell sub-populations. The authors found that the CD44+/CD24- subpopulation of esophageal cancer cells exhibited a higher proliferation rate and sphere forming potential and was more radioresistant *in vitro* than unselected or CD44+/CD24+ cells. In a study of the archival pre-neoadjuvant CRT biopsy material from esophageal AC patients (N = 27), CD44+/CD24- cells could only be identified in 50% (9/18) of poor responders to neoadjuvant CRT, but never (0/9) in complete responders. These results warrant further investigation into the possible clinical utility of CD44+/CD24- phenotype as a predictive biomarker for the response to CRT in patients with esophageal cancer.

Human epidermal growth factor receptors 1 and 2

(EGFR and HER2/neu) are known to be involved in malignant transformation and tumor growth. Yamamoto *et al*[65] assessed the expression of EGFR, HER2/neu, HER3, Ki-67 and p53 by immunohistochemistry in 37 esophageal SCC patients treated with neoadjuvant chemotherapy and found that EGFR expression correlated with pathological response to neoadjuvant chemotherapy. Akamatsu *et al*[66] reported similar findings in 34 patients who had esophageal SCC and were receiving neoadjuvant CRT, *i.e.*, positive staining for HER2/neu was found to be associated with CRT resistance. In contrast, Arsenijevic *et al*[67] and Schena *et al*[68] found no statistically significant difference between EGFR and HER2/neu expression and the clinical response to neoadjuvant CRT. Further verification studies are necessary to clarify the role of EGFR and HER2 expression in the response of esophageal cancer patients to CRT.

The tumor suppressor gene p53, which is involved in cell cycle regulation, apoptosis and DNA repair, has been identified as an important molecular factor in the response to neoadjuvant therapy in patients with esophageal cancer[69]. However, the predictive value of p53 status for chemotherapy response in esophageal cancer patients has not been established. Kitamura *et al*[70] performed a study involving 95 patients with esophageal SCC and showed that p53 protein expression was significantly associated with increased sensitivity to neoadjuvant CRT. In contrast to these findings, Shimada *et al*[71] demonstrated that p53 protein expression was negatively associated with histopathological response to chemotherapy, whereas other similar studies did not find any predictive value for p53 in multimodality therapy for esophageal cancer[67,72]. Zhang *et al*[73] conducted a meta-analysis of 28 studies comprising 1497 cases to elucidate the correlation of p53 status with the response to chemotherapy-based treatment. The authors concluded that patients with low expression of wild-type p53 had higher rates of complete pathological response to neoadjuvant CRT. The clinical significance of p53 as a predictive biomarker for the treatment of esophageal cancer should be further evaluated.

DNA repair pathways are essential for the cell responses to DNA damage induced by CRT. Aberrant regulation of DNA repair proteins is frequently reported in cancers and the reduced expression of these proteins correlated with poor prognosis in esophageal cancers[74-76]. Alexander *et al*[77] assessed major DNA repair proteins such as XPF, FANCD2, PAR, MLH1, PARP1 and phosphorylated MAPKAP kinase 2 in 79 patients with esophageal cancer by tissue microarray. The authors showed that higher scores for MLH1 and lower scores for FANCD2 were significantly associated with pathological response to neoadjuvant CRT on multivariable analysis.

Expression of heat-shock proteins (HSPs) and glucose-regulated proteins (GRPs) can be induced in cells following exposure to different insults, allowing cells to survive stress conditions. The regulation and expression of these proteins have an important impact on the biology of esophageal cancer with respect to prognosis[78] and response to chemotherapy[79]. Slotta-Huspenina *et al*[80] assessed HSPs and GRPs by reverse phase protein arrays (RPPAs), immunohistochemistry and quantitative RT–PCR in pretherapeutic biopsies of 90 patients with esophageal AC. The authors showed that low expression of HSP90, HSP27 and p-HSP27$^{(Ser15, Ser78, Ser82)}$ and high expression of GRP78, GRP94, HSP70 and HSP60 were significantly associated with pathological response to neoadjuvant chemotherapy.

Even with the advances in modern technologies, the emergence of new biomarkers for esophageal cancer has been relatively slow because biomarker discovery has been generally hypothesis-driven and depended on investigation of individual genes or proteins. Data-driven approaches such as global expression studies provide a considerable number of biomarker candidates and once their functional and clinical significance is established, they are worth validating by immunohistochemistry. Immunohistochemistry is an established clinical examination method and further validation studies on biomarker candidates confirmed by immunohistochemistry should be relatively easily performed. A possible utility of these candidate proteins as predictive biomarkers for neoadjuvant CRT should be further validated.

Serum biomarkers with response to treatments

The hypothesis-driven approach is used to examine serum proteins, which have been previously established as biomarkers but have not been considered as predictive biomarker candidates. Serum samples can be obtained by a minimally invasive procedure at a relatively low cost and thus can be repeatedly examined. There are several reports that conventional serum biomarkers could be predictive in esophageal cancer.

Makuuchi *et al*[81] examined the expression levels of 84 cytokines in serum samples obtained from 37 esophageal SCC patients treated with neoadjuvant CRT. They found that the level of serum soluble IL-6 receptor was significantly higher in 30 patients who failed to achieve a complete histological response, thereby revealing a correlation between serum IL-6 receptor levels and the histological response to neoadjuvant CRT. These observations suggest that persistent systemic inflammation can be a possible mechanism of resistance to CRT therapy in esophageal cancers.

Brabender *et al*[82] assessed thymidylate synthetase and dihydropyrimidine dehydrogenase RNA expression in the peripheral blood of 29 patients who had esophageal cancer and had been treated with neoadjuvant CRT. The authors showed that high thymidylate synthetase expression was associated with a minor response to neoadjuvant treatment, while there was no significant association between dihydropyrimidine dehydrogenase and treatment response. They also reported that the specificity of response prediction reached 100% when the levels of thymidylate synthetase and dihydropyrimidine dehydro-

genase were assessed simultaneously.

Only a few serum biomarkers have been examined for predictive utility in cancers and it is challenging to investigate the rest of them. Such an examination does not require significant sample volumes and it is quite feasible to examine multiple serum biomarkers in identical cohorts. Serum biomarkers can be routinely examined in the clinical setting and their application to the prediction of treatment responses seems to be quite promising.

Common blood tests

Data obtained by common blood tests can be an indicator of response to neoadjuvant therapy. It is noteworthy that, although serum examination may lack specificity and sensitivity, its combination with common blood tests can provide predictive stratification of esophageal cancer patients for chemotherapy.

Sato *et al*[83] investigated the correlation between the pre-therapeutic neutrophil to lymphocyte ratio (NLR) and pathological response to neoadjuvant chemotherapy in patients with advanced esophageal cancer. The authors showed that the pretreatment NLR ($< 2.2/ \geqslant 2.2$) was significantly correlated with pathological response: the pathological response rates were 56% and 21% in patients with the NLR < 2.2 and NLR > 2.2, respectively. Similar results were reported by Noble *et al*[84], who examined the correlation of blood-borne inflammatory and nutritional markers with response to neoadjuvant chemotherapy in radically treated esophagogastric cancer patients. The authors demonstrated that only serum albumin ($P = 0.037$) had a predictive value for the pathological response to chemotherapy and that a higher NLR was associated with poor overall survival. In contrast, Hsu *et al*[85] reported that none of the clinical parameters, including blood profiles, images and baseline tumor characteristics, predicted the response to CRT.

Cancer always unfolds on a background of chronic inflammation and it is an interesting idea that inflammatory markers can also serve as prognostic biomarkers for cancer therapy. On the other hand, parameters of systemic inflammation can be confounding factors in a cancer biomarker study. Stricter sample stratification for biomarker studies and extensive independent validation by independent researchers may distinguish true biomarkers from the confounding factors. The results obtained by current studies seem to be promising and further validation will confirm the prognostic utility of candidate biomarkers for clinical applications (Table 1).

TREATMENT STRATEGY BASED ON THE STATUS OF PREDICTIVE BIOMARKERS

As described above, a number of molecules have emerged as predictive candidate biomarkers for the treatment of esophageal cancers and will hopefully result in establishment of biomarkers for routine clinical use. By combining several promising markers in a cross-modality manner, we may be able to develop versatile predictive tools that are more effective than single markers. This approach should be achieved by linking the biomarker components to stratified patient information. The diagnostic kit may be developed such that it gets a local makeover to adjust for variations in clinical therapeutic approaches. The effectiveness of response prediction depends on therapeutic strategies, including the surgical procedure and neoadjuvant therapy, and the clinical background of patients with esophageal cancer. For example, neoadjuvant chemotherapy with cisplatin plus 5-fluorouracil is the current standard treatment for locally advanced esophageal cancer in Japan[86], while neoadjuvant CRT with cisplatin plus 5-fluorouracil is the standard in Western countries[87]. In Japan, a three-arm Phase Ⅲ trial started in November 2012 to confirm the superiority of docetaxel and cisplatin plus 5-fluorouracil over cisplatin plus 5-fluorouracil and the superiority of cisplatin plus 5-fluorouracil with CRT over cisplatin plus 5-fluorouracil as neoadjuvant therapy for esophageal SCC[88]. If neoadjuvant chemotherapy is combined with radiation therapy, the prediction kit should include the biomarkers associated with sensitivity to radiation, such as RNA-binding protein RNPC1[89]. On the other hand, if the combination chemotherapy regimen includes docetaxel, a docetaxel-specific biomarker, such as RPN2[90], should be present. In addition, a predominant histological type of esophageal cancer has been found to exhibit region-dependent differences. Thus, SCC is the predominant histological type of esophageal carcinoma worldwide; however, in Australia, the United Kingdom, the United States, and some Western European countries (*e.g.*, Finland, France, and the Netherlands), the incidence of esophageal AC now exceeds that of SCC[91,92]. In a study on 8562 patients who underwent surgical resection, Merkow *et al*[93] found that the only factor predictive of pathological complete response was SCC histology. The response pattern to neoadjuvant therapy is different in each histological type[94]. Thus, to increase the specificity of response prediction, different molecules can serve as biomarkers depending on histological type. Any article clubbing two diseases together is not appropriate. Surgical procedures are also different in each country. Surgical options for the resection of esophageal carcinoma include the following: trans-hiatal esophagectomy and trans-thoracic approaches, such as Ivor Lewis esophagectomy (abdominal and right thoracic approach also called the Lewis-Tanner approach), the three-incision modified McKeown esophagectomy (involving laparotomy, right thoracotomy, neck anastomosis, and left thoracotomy) and the left thoraco-abdominal approach[95-101]. In Japan and several other countries, extended lymphadenectomy is a common procedure, but this is not the case elsewhere[102-104]. In conclusion, because the sensitivity and specificity of response prediction vary according to regional differences in therapeutic strategies and clinical background, it may be necessary to customize a prediction kit for each country rather than to adopt a universal prediction strategy.

Table 1 Molecular biomarkers for predicting the response to neoadjuvant therapy in esophageal cancer

Modality/biomarker	*N*	Histology	Neoadjuvant therapy	Sensitivity	Specificity	PPV	NPV	Accuracy	Ref.
Gene expression profiling									
5 genes (EPB41L3, RNPC1, RTKN, STAT5B, and NMES1)	13	Squamous-23% Adeno-77%	CRT; 5-FU and cisplatin, 40.05-44 Gy	100%	91%	NA	NA	95%	[15]
3 genes (PERP, S100A2, and SPRR3)	19	Squamous-11% Adeno-84%	CRT; 5-FU, docetaxel and irinotecan, 50.4 Gy	86%	85%	75%	92%	85%	[16]
Ephrin B3 receptor	47	Adeno-100%	CT; 5-FU, cisplatin and leucovorin	89%	84%	89%	84%	87%	[17]
199 genes	25	Squamous-100%	CT; 5-FU, cisplatin and adriamycin	68%	93%	88%	79%	82%	[18]
32 genes	46	Squamous-46% Adeno-54%	CRT; 5-FU and cisplatin, 35-50 Gy	100%	67%	55%	100%	76%	[19]
Single nucleotide polymorphisms									
XRCC1 R399Q	210	Squamous-17% Adeno-83%	CRT; 5-FU, cisplatin and paclitaxel, RT (NA)	NA	NA	NA	NA	NA	[26]
ERCC1 C118T/XRCC1 A194G	52	Squamous-60% Adeno-40%	CRT; 5-FU and cisplatin, 36 Gy	54/5%	67/100%	80/100%	37/59%	58/60%	[27]
MicroRNAs									
miR-192, miR-194	8	Squamous-25% Adeno-75%	CRT; 5-FU and cisplatin, 40 Gy	NA	NA	NA	NA	NA	[40]
HS-240, has-miR-296, has-miR-141, has-miR-31, HS-217	25	Squamous-20% Adeno-80%	CRT; cisplatin and irinotecan, 50.4 Gy	NA	NA	NA	NA	NA	[41]
Serum miR-200c	64	Squamous-100%	CT; 5-FU, cisplatin and adriamycin or docetaxel	68%	62%	53%	75%	64%	[43]
miR-200c	98	Squamous-91%	CT; 5-FU, cisplatin and adriamycin	NA	NA	NA	NA	NA	[44]
miR-31	19	Squamous-5% Adeno-95%	CRT; 5-FU and cisplatin, 40.05 Gy	NA	NA	NA	NA	NA	[45]
Proteomics									
Mitochondrial respiratory chain complexes	69	Adeno-100%	CT; 5-FU and cisplatin	50%	93%	82%	74%	71%	[53]
C4a, C3a	31	Squamous and adeno; NA	CRT; 5-FU and cisplatin, 40-44 Gy	79%	83%	NA	NA	81%	[54]
Immunohistochemistry									
CD44+/CD24-	27	Adeno-100%	CRT; NA	50%	100%	100%	50%	67%	[64]
EGFR	37	Squamous-100%	CT; 5-FU, cisplatin and docetaxel	93%	55%	58%	92%	70%	[65]
HER2/neu	34	Squamous-100%	CRT; 5-FU and cisplatin or leucovorin, 39.6-40 Gy	69%	71%	60%	79%	71%	[66]
p53 (wild-type)	1497	Squamous-91% Adeno-9%	CRT or CT (meta-analysis)	NA	NA	NA	NA	NA	[73]
MLH1, FANCD2	79	Squamous-27% Adeno-71%	CRT; 5-FU, cisplatin and/or paclitaxel, 45-64.8 Gy	20%	100%	100%	22%	35%	[77]
Heat-shock proteins and glucose-regulated proteins	90	Adeno-100%	CT; 5-FU, cisplatin or oxaliplatin	61%	63%	53%	70%	62%	[80]
Serum biomarker									
Serum soluble interleukin-6 receptor	37	Squamous-100%	CRT; 5-FU and cisplatin, 40 Gy	NA	NA	NA	NA	NA	[81]
Thymidylate synthetase and dihydropyrimidine dehydrogenase	29	Squamous-34% Adeno-66%	CRT; 5-FU and cisplatin, 36 Gy	20%	100%	100%	36%	45%	[82]
Common blood tests									
Neutrophil-to-lymphocyte ratio	83	Squamous-84%	CT; 5-FU and cisplatin	71%	66%	56%	79%	68%	[83]
Albumin	246	Squamous-13% Adeno-86%	CT; cisplatin, epirubicin and 5-FU or capecitabine, or epirubicin and oxaliplatin	NA	NA	NA	NA	NA	[84]

PPV: Positive predict value; NPV: Negative predict value; Squamous: Squamous cell carcinoma; Adeno: Adenocarcinoma; CRT: Chemoradiotherapy; CT: Chemotherapy; 5-FU: 5-fluorouracil; NA: Not available.

Pathological nonresponders to neoadjuvant therapy for esophageal cancer demonstrate no survival benefits compared to patients treated with primary esophagectomy[12]. Factors predicting the response to neoadjuvant therapy may help to reduce the number of unnecessarily treated patients and lead to the investigation of new and more effective therapeutic strategies for the unresponsive group. However, if there are no effective therapies for nonresponders, predicting the response to neoadjuvant therapy is tantamount to abandoning nonresponders to their fate. Further improvement in outcomes for the patient with esophageal cancer cannot be

achieved without improvement of the prognosis of nonresponders. Therefore, the development of new effective therapies for nonresponders concurrently with progress in predictive methodology is necessary. Recently, novel therapeutic approaches, such as new targeted strategies, epigenetic therapeutics, monoclonal antibody therapy and carbon-ion radiotherapy, are being developed[105-107]. Although initially many of these studies involved patients with metastatic disease, these therapies are now being increasingly investigated in the preoperative setting as components of multimodality therapy[105]. The efficacy of targeted agents for neoadjuvant therapy of patients with esophageal cancer has yet to be established in previous and ongoing clinical trials[106]. Additional trials to examine new targeted agents have been performed. Further improvement of the prognosis of esophageal cancer patients can be achieved through the introduction of these novel therapeutic approaches in practice, which provides prognostic improvement for nonresponders identified by predictive biomarkers.

CLINICAL APPLICATION OF BIOMARKERS

Advances in modern omics technologies and the integration of the results into clinical practice provide valuable opportunities for biomarker discovery research. As discussed in this review, considerable numbers of promising biomarkers in esophageal cancer have been established and more biomarker candidates are likely to be identified by the application of novel technologies. These biomarkers have been discovered through a hypothesis-driven approach by medical doctors for specific clinical applications and they seem to have great potential in providing benefits to patients. However, only a few of the biomarkers discovered in the last decade have been introduced into clinical practice and skepticism about the clinical utility of biomarkers in the diagnosis and treatment of cancer has been expressed[108]. As discussed here, treatments based on the results of biomarker studies should be further developed to benefit all patient subgroups. To establish the reliability of biomarkers before clinical trials, the reproducibility of the results should be assessed by independent investigators. However, we found that none of the biomarkers reviewed in this article had been validated by other researchers. Small sample sizes may be the most serious obstacle for validation of predictive biomarkers. Although it is generally accepted that multi-institutional and inter-disciplinary collaboration is required for biomarker validation, until now no serious validation studies have been performed for any predictive biomarkers in esophageal cancer and this issue requires further analysis.

CONCLUSION

We have reviewed the current status of biomarkers in esophageal cancer, especially focusing on the utility for predicting responses to neoadjuvant therapy. The reported biomarkers seem to be promising because they have been developed based on clinical research and their predictive performance has been examined by using clinical samples. Further validation and functional evaluation will increase the reliability of these biomarkers. Combined use of the reported biomarkers may increase prognostic performance and this concept is worth further research. Prognostic modalities should be tailored to specific clinical therapeutic approaches that differ according to individual cases. The development of new effective therapies for nonresponders can be hoped for with the progress in predictive techniques. Further understanding of the molecular mechanisms underlying the resistance to CRT in cancers can be achieved by investigating the functional effects of biomarkers on the malignant properties of tumor cells and such efforts will pave the way to novel therapeutic strategies.

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