Review

Novel Candidate Biomarkers of Chemoradiosensitivity in Esophageal Squamous Cell Carcinoma: A Systematic Review

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Esophageal cancer · Squamous cell carcinoma · Chemoradiosensitivity · Biomarker · Prognosis

Abstract

There is no doubt that, along with surgery, chemoradiotherapy is an important treatment for esophageal squamous cell carcinoma (ESCC). Patients who respond well to chemoradiotherapy obtain great benefits toward overcoming their cancer, and so a more favorable prognosis. On the other hand, patients who do not respond well have wasted valuable time and experienced severe toxicity and seriously diminished quality of life, only to have their cancer recur with an unfavorable prognosis. For this reason, a reliable biomarker of chemoradiosensitivity in ESCC has long been sought. In this review, we will enumerate recently reported candidate biomarkers of chemoradiosensitivity in ESCC that have the potential for future clinical application.

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Introduction

According to the WHO, in 2014 esophageal cancer was the 6th most common cancer globally [1]. In fact, thoracic esophageal squamous cell carcinoma (ESCC) is the most common form of cancer in many regions of the world, and is characterized by an extremely poor prognosis and rapid clinical progression [2, 3]. Nonetheless, thanks to recent advances in surgical technique, postoperative management and chemoradiotherapy (CRT), the outcomes of these patients have improved [4–7]. Still, the 5-year survival rate among ESCC patients remains low, and further developments in this field are needed.

There is no doubt that, along with surgery, CRT is an important treatment strategy for advanced ESCC [2–7]. Patients who respond well to CRT obtain great benefits toward over-

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coming their cancer, and so obtain a more favorable prognosis. On the other hand, patients who do not respond well (e.g., when the tumor does not show chemoradiosensitivity) have wasted valuable time and experienced severe toxicity and seriously diminished quality of life, only to have their cancer recur with an unfavorable prognosis [4–8]. For this reason, a reliable marker of chemoradiosensitivity in ESCC has long been sought [8].

In an excellent review, Okumura et al. [9] discussed biomarkers identified between 1980 and 2011 that are potentially predictive of the ESCC response to neoadjuvant CRT. Those authors concluded that p53 is the most important candidate biomarker. However, no biomarker, including p53, has yet been widely accepted for clinical application, nor has one been mentioned as useful for predicting chemoradiosensitivity in the Guidelines for Diagnosis and Treatment of Carcinoma of the Esophagus in Japan [10]. This is because there is still not sufficient evidence of the usefulness of these biomarkers for that purpose.

The purpose of this systematic review article is to enumerate candidate biomarkers reported between 2011 and early 2015 as predictive of chemoradiosensitivity and discuss their potential for future clinical application in ESCC patients.

Methods

Literature Search

A systematic literature search was performed using PubMed and Google Scholar to evaluate the predictive value of novel candidate biomarkers of chemoradiosensitivity in patients with ESCC. The search strategy was to use combinations of the search terms ‘esophageal cancer’, ‘squamous cell carcinoma’, ‘chemoradiotherapy’, ‘chemoradiosensitivity’, ‘prognosis’ and ‘biomarker’ to identify papers published between 2011 and August 25, 2015. The search was performed repeatedly until no new relevant articles were found. The reference lists of the identified studies were then checked for additional relevant studies, including in vitro assays.

Inclusion/Exclusion Criteria

The studies included met the following criteria: (1) patients were diagnosed with ESCC based on pathological examination; (2) the biomarker was examined before treatment; (3) patients were treated with CRT before, after or in the absence of surgery; (4) the relationship between biomarker expression and any prognosis [overall survival (OS), disease-specific survival (DSS) and disease-free survival (DFS)] was evaluated, and (5) full-length studies in English. Exclusion criteria were as follows: (1) reviews, letters, case reports; (2) patients were diagnosed with adenocarcinoma or head and neck cancer based pathological examination, and (3) the biomarker was examined during or after treatment. Overlapping articles were excluded by examining the authors’ names and affiliations in each publication.

Literature Searches and Study Characteristics

The search strategy generated 250 references, including 45 in PubMed and 205 in Google Scholar. Of those, only 32 potentially eligible studies were identified. We excluded no studies due to failure to report available data. We thus included 32 full-length articles that met the inclusion criteria. The selection process used for the articles is illustrated in the PRISMA 2009 flow diagram shown in figure 1. We classified the biomarkers based on the testing approach used by the authors, ‘blood examination’ or ‘tissue examination’. We further classified the biomarkers such as ‘positive biomarker’, ‘negative biomarker’ and ‘combination of biomarkers’ in both testing approaches. The classified candidate biomarkers are summarized in tables 1 and 2.

Positive Biomarkers in Blood Examination

Insulin-like growth factor binding protein-3 (IGFBP-3) is a key regulatory molecule in the IGF axis and functions in a tissue-specific way as both a tumor suppressor and promoter [11]. Zhao et al. [12] reported that ESCC patients carrying the IGFBP-3 rs2854744 GG or GC

Fig. 1. The selection process used for the articles is illustrated in the PRISMA 2009 flow diagram.

genotype have a significantly better response to CRT and longer OS than those with the CC genotype. On the other hand, we previously reported that IGFBP-3 is highly expressed in ESCC cells deemed highly susceptible to radiotherapy based on cDNA microarray analysis [13]. Moreover, knocking down IGFBP-3 expression in radiosensitive ESCC cells using siRNA significantly reduced radiosensitivity. However, the relationship between the IGFBP-3 SNP and its expression in ESCC remains to be clarified.

Tumor necrosis factor- α (TNF- α), an important proinflammatory cytokine, is produced by activated macrophages. TNF- α and its superfamily members are well known to have both beneficial and harmful effects. Although TNF- α was discovered as a cytokine able to kill tumor cells, it can also stimulate cell proliferation, invasion and metastasis [14]. Omatsu et al. [15] found that the TNF- α -857C>T genotype is predictive of long-term survival in Japanese ESCC patients treated with definitive CRT.

Table 1. Novel candidate biomarkers in blood that are predictive of chemoradiosensitivity in ESCC

Biomarker	Major function	Sample size	Method	Predictive value of the sensitivity	First author [Ref.]	Year
<i>Positive</i>						
IGFBP-3	IGF transport	110	blood PCR	rs2854744 GG + GC genotype	Zhao [12]	2015
TNF- α	cell apoptosis	46	blood PCR	TNF- α 857C>T	Omatsu [15]	2013
Hemoglobin	oxygen transport	123	blood exam	Hb >13 g/dl	Hamai [16]	2014
Albumin	colloidal osmotic pressure	74	blood exam	Alb >3.5 g/dl	Hamai [17]	2013
<i>Negative</i>						
VEGF	tumor angiogenesis	79	serum PCR	VEGF-A(-), TGF- β_1 (-)	Cheng [19]	2014
		49	blood PCR	VEGF 634 C/G	Tamura [20]	2012
XPA, XPC	DNA repair	501	blood PCR	XPA 5'UTR, XPC K939Q wild type	Yang [23]	2013
IL-6R	cytokine receptor	64	serum PCR	IL-6R (-)	Makuuchi [25]	2013
<i>Combination</i>						
Octanoylcarnitine, lysoPC(16:1), decanoylcarnitine	metabolomics	44	RRLC-MS	octanoylcarnitine (-), lysoPC(16:1) (-), decanoylcarnitine (-)	Xu [27]	2013

RRLC-MS = Rapid resolution liquid chromatography-mass spectrometry.

Hemoglobin is one of the most routinely assayed blood components in clinical practice. However, a multivariable analysis performed by Hamai et al. [16] revealed that the pretreatment hemoglobin level is an independent predictor of the response to neoadjuvant CRT, and that patients with hemoglobin levels <13 g/dl had a significantly higher rate of poor responders and worse 5-year OS. Those investigators also reported in a separate study that a pretreatment serum albumin level >3.5 g/dl was independently associated with prolonged survival [17].

Negative Biomarkers in Blood Examination

Vascular endothelial growth factor (VEGF)-A is a potent signaling molecule that contributes to tumor angiogenesis and hematogenous spread of disease [18]. Cheng et al. [19] demonstrated that among ESCC patients treated with neoadjuvant CRT followed by esophagectomy, those exhibiting high serum levels of VEGF-A and transforming growth factor (TGF)- β_1 had a significantly worse pathological response and worse DSS. Additionally, through analysis of peripheral blood from 49 Japanese patients with ESCC, Tamura et al. [20] found that the VEGF-634C/G genotype [an SNP in the 5' untranslated region (UTR)] is predictive of better OS after treatment with definitive CRT. It has also been reported that genetic polymorphisms in the promoter region, 5'UTR and 3'UTR of the VEGF gene, are associated with VEGF levels in plasma and tumors [21], which suggests the VEGF-634C/G genotype may be related to the blood VEGF level.

XPA and XPC are important molecules involved in mediating nucleotide excision for DNA repair [22]. Yang et al. [23] used polymerase chain reaction (PCR) to analyze the genomic DNA isolated from peripheral leukocytes collected from 501 ESCC patients and demonstrated that patients with the XPA 5'UTR A/G and XPC K939Q C/C genotypes are at higher risk of mortality after treatment than patients with wild-type homozygous genotypes, and this effect was more pronounced in patients treated with neoadjuvant CRT followed by esophagectomy.

Table 2. Novel candidate biomarkers in tissue that are predictive of chemoradiosensitivity in ESCC

Biomarker	Major function	Sample size	Method	Predictive value of the sensitivity	First author [Ref.]	Year
<i>Positive</i>						
ZNF695	DNA repair	104	PCR	ZNF695 methylation (+)	Takahashi [28]	2015
LOC285194	long noncoding RNA	55	PCR	LOC285194 (+)	Tong [31]	2014
REG1A	cell proliferation	105	IHC	REG1A (+)	Sato [35]	2013
<i>Negative</i>						
MDM2	oncogene	79	IHC	MDM2 (–)	Okamoto [38]	2015
Nrf2	intracellular antioxidants	46	IHC	Nrf (–)	Kawasaki [41]	2014
		33	PCR	Nrf mutation (–)	Shibata [42]	2011
Rad51	DNA repair	39	IHC	Rad51 (–)	Nakanoko [44]	2014
HIF-1 α	homeostatic response to hypoxia	942	meta-analysis	HIF-1 α (–)	Ping [46]	2014
PC4	DNA repair	98	IHC	PC4 (–)	Qian [48]	2014
ERCC1	DNA repair	44	IHC	ERCC1 (–)	Ge [50]	2014
EGFR	cell proliferation	47	IHC	EGFR (–)	Gao [54]	2014
		148	PCR	EGFR homozygous long allele (L/L)	Lee [55]	2011
PITX2	embryonic development	424	IHC	PITX2 (–)	Zhang [58]	2013
PinX1	telomere stability	157	IHC	PinX1 (–)	Qian [60]	2013
BRCA1	DNA repair	144	PCR	BRCA1 (–)	Gao [64]	2013
p53	tumor suppressor	1,497	meta-analysis	p53 low or wild type	Zhang [65]	2013
mTOR	cell proliferation	77	IHC	mTOR (–)	Li [67]	2012
COX-2	PG synthesis	58	IHC	COX2 (–)	Akutsu [72]	2011
<i>Combination</i>						
MMP1, LIMCH1, C1orf226	ECM degradation	60	PCR	MMP1 (+), LIMCH1 (–), C1orf226 (–)	Wen [75]	2014
Ki-67, NF- κ B, HER2, ER	cell proliferation	10	IHC	Ki-67 (+), NF- κ B (–), HER2 (–), ER (–)	Shibata [78]	2013
MLH1, FANCD2, PARP1, XPF, PAR	DNA repair	79	IHC	MLH1 (+), FANCD2 (–)	Alexander [82]	2012
PTCH1, GLI-1	development of organs	100	IHC	PTCH (–), GLI-1 (–)	Zhu [84]	2011

IHC = Immunohistochemistry; ECM = extracellular matrix; PG = prostaglandin.

Interleukin-6 receptor (IL-6R; also known as CD126) is a cell membrane cognate receptor for the inflammatory cytokine IL-6. Soluble (s)IL-6R is an alternatively spliced or proteolytically cleaved form of IL-6R that lacks the transmembrane domain. Secreted sIL-6R binds to IL-6, and the resultant sIL-6R/IL-6 complex evokes intracellular signaling [24]. Makuuchi et al. [25] showed that the serum sIL-6R levels were significantly higher in 30 patients who failed to achieve a pathological complete response (pCR), and multivariate analysis indicated that a high sIL-6R level is one of several significant independent predictors of an unfavorable outcome after preoperative CRT followed by esophagectomy.

Combination of Biomarkers in Blood Examination

Metabolomics is a powerful approach that enables quantitative measurement of global changes in the metabolic profiles of individuals in response to disease or treatment through noninvasive analysis of biofluids [26]. Using metabolomics, Xu et al. [27] observed that the

levels of three metabolites, lysoPC(16:1), octanoylcarnitine and decanoylcarnitine, correlated closely with treatment and showed a tendency toward gradual recovery to healthy levels in the responder group. In the nonresponder group, no obvious changes in the levels of these metabolites were observed over the treatment course. It was therefore concluded that these biomarkers may be useful in diagnosis, as well as for monitoring therapeutic responses and predicting outcomes in ESCC.

Positive Biomarkers in Tissue Examination

DNA methylation status reportedly correlates with the clinicopathological features of many types of cancer. Using genome-wide methylation analysis with 7 responders and 8 nonresponders, Takahashi et al. [28] showed that ZNF695 methylation in ESCC tissue samples is an independent factor predictive of the response to definitive CRT. Because ZNF695 is a zinc finger protein and a likely transcription factor, they speculated that ZNF695 is predictive of outcome because it regulates the expression of genes involved in the repair of DNA damage introduced by CRT. Recently, it was reported that ZNF695 splice variants were highly expressed in ovarian cancer [29]. Although it is still unknown whether these splice variants are associated with aggressive behavior in ovarian cancer, it is possible that through loss of ZNF695 function, cancer cells become highly proliferative, resulting in resistance to therapy. There are no other reports of a relationship between ZNF695 and cancer.

Long noncoding RNAs (lncRNAs) represent a new class of non-protein-coding RNAs, which are longer than 200 bases and do not function as templates for protein synthesis. Studies have also shown that lncRNAs play a critical role in the development and progression of cancers, including ESCC [30]. Tong et al. [31] reported that low LOC285194 expression correlated significantly with a poorer CRT response and that patients with low LOC285194 expression had significantly shorter DFS and OS. Moreover, multivariable analysis identified low LOC285194 expression as an independent prognostic factor for CRT response, DFS and OS. Though numerous reports on the relationship between lncRNAs and esophageal cancer have been published, there are no lncRNA candidates other than LOC285194 whose expression relates to chemoradiosensitivity.

REG1A was originally isolated from pancreatic islet β -cells as an endogenous growth factor [32]. We previously reported that REG1A is a reliable sensitivity marker in ESCC patients (clinical T4) treated with neoadjuvant CRT [33] or definitive CRT [34]. We also recently showed that among stage II and III ESCC patients treated with esophagectomy followed by adjuvant chemotherapy, OS, DSS and DFS were all significantly better in REG1A-positive than REG1A-negative patients [35]. Additionally, we demonstrated that REG1A gene transfection enhances radiosensitivity by affecting the MAPK pathway in ESCC cells [36]. New studies aimed at determining whether REG1A also works as a reliable sensitivity marker in stage II and III ESCC patients treated with neoadjuvant CRT followed by esophagectomy are now ongoing. However, the number of patients in our institution is limited, so accumulation of strong evidence will take time. Studies of REG1A from other institutions treating different populations will facilitate clinical application of REG1A as a reliable sensitivity marker in ESCC.

Negative Biomarkers in Tissue Examination

MDM2 promotes tumor formation by targeting tumor suppressor genes (e.g., p53 and p16), and its overexpression has been detected in a variety of different cancers [37]. Okamoto et al. [38] reported that MDM2 positivity in pretreatment biopsy specimens was significantly

higher in the failure group than in the CR group among cStageIII ESCC patients treated with definitive CRT. In addition, it was reported that the MDM2 inhibitor Nutlin-3 increased the radiosensitivity of laryngeal squamous cell carcinoma [39]. Therefore, it may be clinically worthwhile to attempt a clinical study of this inhibitor in patients with ESCC.

Nrf2 was originally identified as a critical regulator of intracellular antioxidants, and recent studies have shown that strong Nrf2 expression gives cancer cells a survival advantage during anticancer chemotherapy and radiotherapy [40]. Kawasaki et al. [41] found a significant correlation between Nrf2 expression in biopsy specimens before CRT and an unfavorable response to neoadjuvant CRT. In addition, Shibata et al. [42] showed that Nrf2 gene mutation was associated with tumor recurrence and a poor prognosis, and that downregulation of the mutant Nrf2 increases the sensitivity of ESCC cells to 5-fluorouracil and radiation. Strong Nrf2 expression and its gene mutation thus appear to indicate resistance to CRT.

Rad51 plays an important role in the repair of DNA double-strand breaks through homologous recombination, thereby decreasing sensitivity to chemotherapy and radiotherapy [43]. Nakanoko et al. [44] reported that a pCR was more frequently observed in Rad51-negative than Rad51-positive ESCC patients treated with neoadjuvant CRT followed by esophagectomy. Rad51 also interacts with a variety of tumor suppressor proteins, including p53, BRCA1 and BRCA2, indicating a possible association of Rad51 with the process of tumorigenesis and resistance to CRT. However, the role of Rad51 in these processes is not yet understood.

HIF-1 α is a key transcription factor that functions in response to hypoxic stress by regulating genes involved in maintaining oxygen homeostasis [45]. Recently published studies on the prognostic value of HIF-1 α overexpression in ESCC are conflicting and heterogeneous. However, Ping et al. [46] performed a meta-analysis that included 12 studies with a total of 942 ESCC patients and found that HIF-1 α overexpression was a potent predictor of a poor prognosis and resistance to CRT in ESCC. At present, HIF-1 α appears to be one of the most promising candidate markers predictive of the response to CRT in ESCC.

Human positive cofactor 4 (PC4) plays important roles in gene transcription and replication, DNA damage repair, chromatin organization and cell cycle progression [47]. Qian et al. [48] showed that high expression of PC4 positively correlates with resistance to CRT and is an independent predictor of short DSS in ESCC patients who receive definitive CRT with cisplatin (CDDP)-based chemotherapy. However, there are no other reports on the relationship between PC4 and ESCC at this time.

ERCC1 appears to be involved in repairing DNA damage induced by chemotherapy and radiotherapy in cancer tissue [49]. Ge et al. [50] reported that low ERCC1 expression was an independent predictor of better OS in patients with locally advanced ESCC treated with neoadjuvant CRT followed by esophagectomy, and those findings have been confirmed by others [51, 52].

Diffuse epidermal growth factor receptor (EGFR) expression is reportedly associated with reduced OS in patients with locally advanced ESCC [53]. In addition, Gao et al. [54] found a significant correlation between EGFR overexpression and poor OS among patients with locally advanced ESCC receiving definitive CRT, while Lee et al. [55] observed that the EGFR genotype is predictive of clinical outcome after neoadjuvant CRT followed by esophagectomy. When the accumulating evidence becomes sufficient, EGFR-TKI may also be considered for clinical application as a treatment option for ESCC as well as non-small cell lung cancer.

It is well known that PITX2 is crucial for normal embryonic development [56]. In recent studies, PITX2 was identified as a target gene in the Wnt/ β -catenin and hedgehog/TGF pathways, which regulate several oncogenes, including MYC, cyclin D1 and cyclin D2 [57]. Zhang et al. [58] found that strong PITX2 expression was more frequently observed in CRT-resistant patients than in those responsive to CRT, and that strong PITX2 expression was associated with poor DSS among ESCC patients treated with definitive CRT. They also demon-

strated that PITX2 knockdown substantially increased the sensitivity of ESCC cells to radiation or CDDP *in vitro*. However, there are currently no other reports on the relationship between PITX2 and responsiveness to CRT or prognosis.

The telomerase-/telomere-interacting protein PinX1 contributes to telomere maintenance and tumorigenicity, and several studies have documented that telomerase activity is a determinant of cytotoxic drug sensitivity in ESCC [59]. Qian et al. [60] showed that strong expression of PinX1 correlated positively with ESCC resistance to CRT and was a strong and independent predictor of short DSS in ESCC patients. Moreover, PinX1 knockdown substantially increased the therapeutic efficacy of radiation against ESCC cells *in vitro* and *in vivo*, and ectopic overexpression of PinX1 dramatically enhanced ESCC cell resistance to radiotherapy. On the other hand, Zuo et al. [61] reported that PinX1 had the opposite effect on carcinogenesis among esophageal epithelial cells.

BRCA1 was first identified as one of the genes that conferred genetic predisposition to early-onset breast and ovarian cancer [62]. However, increasing numbers of reports have shown that BRCA1 is also a regulator of chemotherapy-induced DNA damage in cancer cells [63]. Gao et al. [64] reported that low BRCA1 transcription correlated with a higher response rate and longer OS in ESCC patients receiving CDDP-based chemotherapy or CRT. On the other hand, low BRCA1 transcription also correlated with shorter OS in patients receiving docetaxel-based chemotherapy or CRT. BRCA1 may thus be an indicator that enables us to appropriately select CDDP-based or docetaxel-based therapy for more individualized treatment.

Though p53 is reportedly useful for predicting the response to CRT in ESCC patients, it remains controversial. In 2013, Zhang et al. [65] reported a meta-analysis that included 28 studies involving 1,497 ESCC patients. They reported that low expression of the p53 protein and/or the wild-type p53 gene was associated with a good response to both chemotherapy and CRT in ESCC. Additionally, patients carrying the wild-type form of p53 had a high rate of pCR to neoadjuvant CRT. These results provide further evidence that p53 would be a reliable biomarker of a positive response to CRT in ESCC.

Clinicians are beginning to use mTOR inhibitors (e.g., temsirolimus and everolimus) in the treatment of cancer as well as several age-associated diseases, including neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [66]. Li et al. [67] showed that levels of phosphorylated mTOR are independently associated with the response to CRT and prognosis in patients treated with neoadjuvant CRT followed by esophagectomy. They also demonstrated that mTOR inhibition sensitizes ESCC cells to chemotherapy *in vitro*. Two other reports have confirmed the connection between mTOR inhibition and improved chemosensitivity in ESCC [68, 69]. Notably, the mTOR pathway leads to upregulation of HIF-1 α , the effects of which have been discussed above [70].

COX1 and COX2 play crucial roles in prostaglandin synthesis. COX1 is expressed in normal tissues to maintain homeostatic function. COX2, by contrast, is primarily expressed in cancers, including ESCC [71], and Akutsu et al. [72] found that COX2 expression is predictive of resistance to neoadjuvant CRT in ESCC patients. In addition, Okamura et al. [73] showed that ESCC cells transfected with COX2 were resistant to CDDP + 5-fluorouracil treatment.

Combination of Biomarkers in Tissue Examination

MMP1 is a member of the matrix metalloproteinase (MMP) family and is reportedly involved in the response to cancer drug therapy through its regulation of vascular leakage [74]. Wen et al. [75] analyzed gene expression in pretreatment biopsy specimens from 28 ESCC patients who received neoadjuvant CRT and esophagectomy. The gene profiling identified 10 differentially expressed genes with more than a twofold difference between patients

with a pCR and those with less than a pCR. Using a combination of 3 genes, MMP1, LIMCH1 and C1orf226, ESCC patients could be effectively and reproducibly classified with respect to their responsiveness to neoadjuvant CRT.

Ki-67 (MIB-1) monoclonal antibody recognizes proliferating cells in the G1, S, G2 and M phases of the cell cycle [76]. The correlation between a high MIB-1 index and poor prognosis is well known in breast cancer [77]. By contrast, Shibata et al. [78] reported that ESCC patients with a higher MIB-1 index showed significantly better OS following definitive CRT than patients with a lower index. It was also reported from a study of 56 ESCC patients that overexpression of MIB-1 is a significant factor for a complete endoscopic response following definitive CRT [79]. The correlation between MIB-1 expression and a good response to CRT may be explained by the fact that MIB-1 expression is a marker of cellular proliferation.

NF- κ B is a protein complex that controls DNA transcription. Blocking NF- κ B appears to cause tumor cells to stop proliferating or become more sensitive to antitumor agents [80]. HER2 is a member of the EGFR family that is involved in the complex regulation of cell growth, proliferation and survival. In addition to breast cancer, HER2 overexpression and gene amplification have also been reported in several other cancers, including ESCC [81]. Estrogen promotes tumor cell proliferation by acting via the estrogen receptor, and that proliferation appears to be enhanced in tumor cells overexpressing HER2. Shibata et al. [78] also showed that low levels of NF- κ B, HER2 and estrogen receptor were good prognostic factors following definitive CCRT.

Alexander et al. [82] analyzed other candidate biomarkers selected from multiple DNA repair pathways. They showed that the expression patterns of DNA repair proteins, including XPF, FANCD2, PAR, MLH1 and PARP1, are associated with the pathological response to neoadjuvant CRT and OS. ESCC patients with high MLH1 and low FANCD2 expression tended to have better OS, but the effect did not reach statistical significance due to the small number of patients.

The hedgehog signaling pathway plays a critical role in the development of various organs, and aberrant activation of this pathway was recently observed in several cancers, including ESCC [83]. Zhu et al. [84] showed that the expression status of two hedgehog components, PTCH1 and the glioma-associated oncogene GLI-1, were independent prognostic factors associated with poor DSS and OS.

Problems of Novel Candidate Biomarkers

In this systematic review, we included 32 recently reported studies and enumerated 41 candidate biomarkers that are potentially predictive of chemoradiosensitivity in ESCC. We found meta-analyses that analyzed 12 studies of HIF-1 α that included a total of 942 patients [46] and 28 studies of p53 that included a total of 1,497 patients [65]. At present, HIF-1 α and p53 appear to be the most promising candidate biomarkers predictive of chemoradiosensitivity in ESCC. However, for even these candidate biomarkers to be useful in clinical application, additional strong evidence of their efficacy will be required. For example, HIF-1 α overexpression was significantly associated with poor OS and DFS, and with a poor response to CRT in a meta-analysis [46]. However, HIF-1 α overexpression was also significantly associated with stage, lymph node metastasis, depth of invasion, lymphatic invasion and distant metastasis, and it is to be expected that more advanced ESCCs are related to a poor outcome and poor response to CRT. Therefore, subgroup analyses among different T statuses, N statuses, M statuses and stages, as well as additional results from large prospective studies performed with different populations will be needed to confirm the reliability of HIF-1 α as an independent sensitivity marker suitable for clinical application.

There are 9 articles on candidate biomarkers in blood, samples of which are easily collected and analyzed using blood PCR, serum PCR and rapid resolution liquid chromatography-mass spectrometry. Blood-borne biomarkers appear to reflect global changes in the body containing ESCC tissue. It is thus widely expected that additional evidence for candidate biomarkers in blood will be produced in the future. On the other hand, there are problems that must be overcome before pretreatment biopsy tissue, which is commonly used today, can be used to predict CRT outcomes. This is because biopsy samples show only a small fraction of an ESCC tumor, and they are quite heterogeneous.

Conclusion

Some of the candidate biomarkers named in this review have been studied at different institutions in different populations, but are still a long way from clinical application. Although it is important to continue to search for novel candidates, it will also be important to continue to obtain strong evidence to determine the suitability for clinical application of candidates already identified.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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