Review Article

The mechanisms of radioresistance in esophageal squamous cell carcinoma and current strategies in radiosensitivity

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Abstract: Esophageal cancer is the eighth most common cancer and the sixth leading cause of cancer-related death worldwide. Surgery is the primary form of treatment, but the survival is poor, especially for patients with locally advanced esophageal cancer. Radiotherapy has been a critical treatment option that may be combined with chemotherapy in patients with unresectable esophageal cancer. However, resistance to chemoradiotherapy might result in treatment failures and cancer relapse. This review will mainly focus on the possible cellular mechanisms and tumor-associated microenvironmental (TAM) factors that result in radioresistance in patients with esophageal cancer. In addition, current strategies to increase radiosensitivity, including targeted therapy and the use of radiosensitive biomarkers in clinical treatment, are discussed in this review.

Keywords: Esophageal cancer; radioresistance; tumor-associated microenvironment (TAM); targeted therapy; radiosensitive biomarker

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Introduction

Esophageal cancer is the eighth most common cancer worldwide. In 2015, approximately 18,170 people were diagnosed with esophageal cancer, and 15,450 people died of the disease in the US (1). Esophageal cancers are divided into two histological groups: squamous cell carcinoma (SCC) and adenocarcinoma (AC). SCC is common in Asia, especially in China, while AC is common in North America and in Western countries. SCC accounts for up to 90% of all esophageal cancers, but the incidence of AC has surpassed that of SCC in North America and Western countries, especially in white men compared with white women (2). Barrett's esophagus is recognized as a precursor lesion of AC, which primarily originates in the lower third of the esophagus (3). Radiotherapy has become an important treatment modality, especially for those patients with unresectable esophageal cancer. Free radicals produced by ionizing radiation may directly affect the DNA or they may indirectly affect other cellular molecules, especially H_2O. These radicals induce the formation of reactive oxygen species (ROS) and subsequent oxidative stress (4). However, resistance to radiation results in relapse and treatment failure. Modalities for the improvement of radiosensitivity are urgently needed for clinical application. The effect of radiotherapy alone is limited, but concurrent chemoradiotherapy and targeted therapy can significantly improve survival rates and control local-regional recurrence

in patients with esophageal cancer.

Cellular mechanisms of radioresistance

Cell cycle checkpoint regulation

The cell cycle checkpoint signaling pathway is a critical process that protects cancer cells from DNA damage. ATM is a phosphatidylinositol kinase-related protein that modulates cell cycle checkpoints after DNA damage is induced by ionizing radiation. Activation of ATM results in dimer dissociation, autophosphorylation and the phosphorylation of downstream proteins including p53, CHK2, and RAD9, among others. Cells are often blocked in the G1/S or G2/M phases, which provides time for cells to repair DNA double-strand breaks (DSBs). Activation of the Cyclin E/CDK2 complex controls G1/S transition through the p53/p21 pathway (5). p53 is a key cell cycle checkpoint regulatory protein that induces G1/S arrest through the activation of p21, which belongs to the Cip/Kip family of CDK inhibitors. Activation of the Cdc2/Cyclin B complex controls G2/M transition through the CHK2 pathway (6). Radiation-induced G1/S arrest prevents the replication of damaged DNA and subsequent entry into S phase, while G2/M arrest prevents the segregation of aberrant chromosomes prior to entry into M phase. Apoptosis is induced to remove the damaged cells if the damage is irreversible or if the phase is dysfunctional. In short, radiation-induced cell cycle checkpoint signaling pathways protect cells from radiation damage and promote the survival of cancer cells. Numerous studies have demonstrated that abrogation of the G2 checkpoint enhances the radioresponse in esophageal cancer cells. Qin *et al.* revealed that the small molecule inhibitor YM155 enhances radiosensitivity through the abrogation of the G2 checkpoint and the suppression of homologous recombination repair in esophageal SCC (7). Che *et al.* found that the COX-2 inhibitor NS398 enhances radiosensitivity in radioresistant esophageal cancer ECA109R50Gy cells through redistribution of the cell cycle, inhibition of expression of the catalytic subunit of DNA-dependent protein kinases and induction of tumor cell apoptosis (8).

Cancer stem cells (CSCs)

Esophageal cancer stem cells (ECSCs) are populations of esophageal cancer cells that possess stem cell properties and that can promote the initiation of tumors whose cells have the ability to self-renew. CD44, CD71, CD90, CD133, CD271, aldehyde dehydrogenase (ALDH), and ATP-binding cassette subfamily G member 2 (ABCG2) have been reported as potential cell surface markers of ECSCs (9-11). The mechanisms by which ECSCs become radioresistant are as follows: (I) DNA repair. The DNA DSBs that occur following radiation are mainly repaired by nonhomologous end joining (NHEJ), which involves repair and recognition by genes such as ATM, XECC4, Ligase 4 and DNA-PKcs. Accumulating evidence has revealed that the ATM signaling pathway is more active in CSCs than in normal cancer cells. Chen *et al.* isolated ECSC, as a side population (SP), from normal esophageal cancer (EC9706 cells) and found that ECSCs avoided apoptosis through a decrease in DNA damage and an increase in DNA damage repair (12). Qian *et al.* found that human positive cofactor 4 (PC4) plays a critical role in NHEJ and DNA damage repair and that the knockdown of PC4 increases apoptosis and mitotic catastrophe (MC) induced by radiation in esophageal SCC (13); (II) cycle distribution. The radiosensitivity of esophageal cancer cells changes as they progress through the different cell cycle phases. Cells exhibit more radiosensitivity in the mitotic phase and more radioresistance in late S phase. CSCs are common in the cell cycle phase in which cells are quiescent. Following radiation, checkpoint kinases activate the ATM and ATR signaling pathways in CSCs to a greater extent than in normal esophageal cancer cells; (III) free radical and ROS scavenging. CSCs can decrease the level of ROS following radiation through the activation of ROS scavenging enzymes such as superoxide dismutase (SOD) and glutathione (GSH). GSH is an intracellular antioxidant molecule whose synthesis is catalyzed by the regulatory subunit of the glutamate-cysteine ligase. As a glutamate-cysteine ligase inhibitor, buthionine sulfoximine (BSO) decreases the colony formation ability of CSCs and increases the antioxidant ability and radiosensitivity of CSCs (14). The activation of markers of radiosensitivity, such as the transcription factors Nrf2 and nuclear factor κB (NF-κB), improves the potency of ROS scavenging enzymes. (IV) Interaction with the stromal microenvironment. Resident fibroblasts secret transforming growth factor β (TGF-β) and promote epithelial-mesenchymal transition (EMT) in CSCs, which could decrease radiosensitivity. CD44 is an extracellular matrix receptor that is expressed on the surface of CSCs and is related to the degree of malignancy.

EMT

EMT is a process through which epithelial cells acquire mesenchymal properties during embryonic development and cancer progression. EMT is characterized by the loss of the epithelial marker E-cadherin and the acquisition of mesenchymal markers including N-cadherin and Vimentin, among others. E-cadherin is a cell adhesion molecule that plays a critical role in the maintenance of epithelial structure. Repression of E-cadherin is the key step in EMT, and this progression may be modulated by the zinc finger proteins Snail and Slug. EMT has been reported to be associated with poor prognosis and chemoradioresistance in numerous malignances. In addition, irradiation might promote the migration and invasiveness of esophageal cancer cells through the EMT process. He *et al.* developed a radioresistant esophageal cancer cell line (KYSE-150RR) via fractional radiation and found that radiation-induced EMT occurred primarily through the PTEN-dependent Akt/Snail signaling pathway (15).

Multiple pro-survival and pro-proliferation signaling pathways

Aberrant Wnt/β-catenin signaling can lead to chromosomal instability and tolerance of DNA damage through its regulation of the mitotic spindle (16). The Wnt signaling pathway downregulates the level of the antiapoptotic gene Bcl-2 as well as the levels of phospho-Akt and upregulates the proapoptotic gene Caspase-3 to drive normal stem cells to become CSCs. Epidermal growth factor receptor (EGFR) and G-protein-coupled receptors activate the PI3K-Akt-mTOR signaling pathway to promote tumor cell growth, proliferation and survival via the inhibition of apoptosis (17). JAK or Src tyrosine kinase activates STAT3, which functions in tumor cell proliferation, differentiation and survival. Autophagy, as a conserved process, mediates the degradation of dysfunctional organelles and the turnover of long-lived proteins and limits the effect of radiotherapy through its support of metabolic mechanisms in conditions of cellular stress (18). Su *et al.* revealed that FH535 increases the radiosensitivity of radioresistant esophageal cancer KYSE-150 cells (KYSE-150R) through a reversal of the expression of Wnt/beta-catenin signaling pathway proteins (Wnt 1, FZD1-4, GSK3β, CTNNB1 and Cyclin D1) (19) (*Figure 1*).

Tumor-associated microenvironment (TAM) and radioresistance

Hypoxia and the HIF-1 pathway

Hypoxia, as a primary mechanism of resistance to radiotherapy and a pathophysiologic characteristic of malignant tumors, interferes with the repair of DNA damage (20). Cancer cell hypoxia often results from the fast rate of tumor growth and when tumors require more than the limited distribution of oxygen within blood vessels (21). At the same time, abnormal angiogenesis and poor vascular function also result in reduced oxygen tension (22). Accumulating evidence from radiation biology and oncology studies has revealed that cancer cells under hypoxic conditions are approximately 2–3 times more radioresistant than those under normal conditions. Radiosensitivity is slowly reduced when the pressure of O_2 is less than 30 mmHg, while cells are maximally radioresistant when the pressure is less than 0.5 mmHg (23). HIF-1, an important transcription factor, induces the expression of multiple genes associated with cellular metabolism, metastasis of tumor cells and angiogenesis. HIF-1, as a heterodimeric factor, contains an α-subunit (HIF-1α) and a β-subunit (HIF-1β). Under normal oxygen conditions, HIF-1α is rapidly degraded through hydroxylation by prolyl hydroxylases (PHDs) and is ubiquitinated by a pVHL-containing E3 ubiquitin ligase. However, under hypoxic conditions, HIF-1α remains stable (24). Using optical imaging, Harada *et al.* revealed that ionizing radiation can activate HIF-1α through a HIF-1α-dependent gene. In esophageal cancer, radiation upregulates the expression of HIF-1α through an improvement in oxidative stress and an increase in the availabilities of glucose and oxygen. Subsequently, HIF-1α increases the expression of VEGF, which protects vascular endothelial cells against the cytotoxic effects of radiation (25). Yang *et al.* demonstrated that berberine enhanced the radiosensitivity of esophageal cancer via the inhibition of VEGF and HIF-1α *in vitro* and *in vivo* (26). Zhu *et al.* found that recombinant human endostatin could enhance the radiosensitivity of esophageal SCC via the downregulation of the expression of VEGF and HIF-1α after radiation therapy and via normalization of the tumor vasculature (27).

Cancer-associated fibroblasts (CAFs)

CAFs have been reported to be abundant in the stroma in

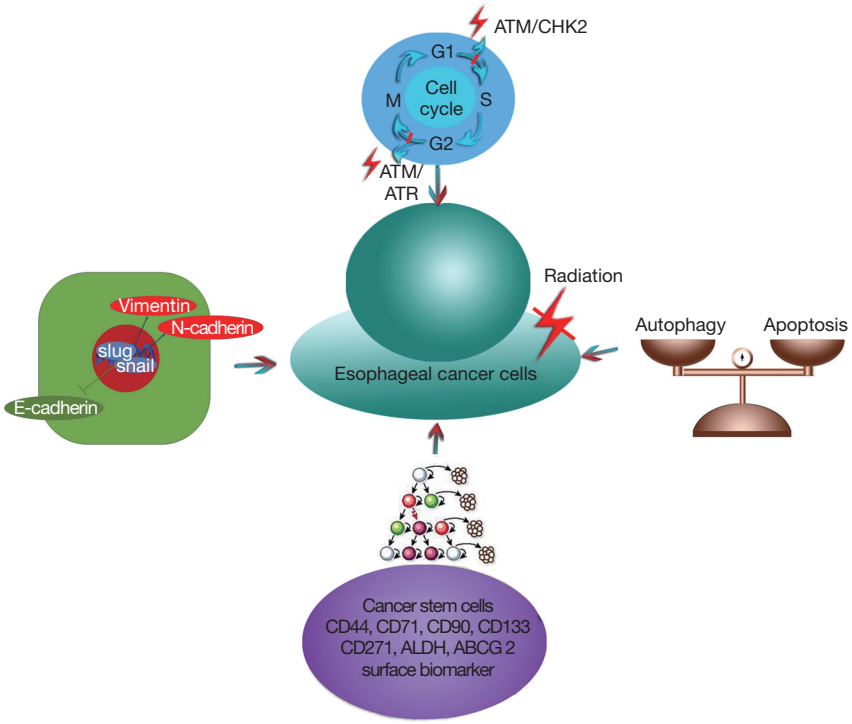

Figure 1 Cellular mechanisms of radioresistance: (I) the cell cycle checkpoint signaling pathway is a critical progress that allowing times for cells to response to repair DNA damage. Cell cycle often arrests in G1/S or G2/M period. The primary mechanism of reparation of DNA double-strand breaks (DSBs) is Nonhomologous end joining (NHEJ); (II) the surface biomarkers of cancer stem cells (CSCs) consist of CD44, CD71, CD90, CD133, CD271, ALDH, and ABCG2; (III) the balance of autophagy and apoptosis is another mechanism of radioresistance. Autophagy, as a conserved process that mediate the degradation, dysfunctional organelles and turnover of long-lived proteins, can limit the effect of radiotherapy through supporting metabolic mechanism in cellular stress times; (IV) epithelial-mesenchymal transition (EMT) is common in cancer progression and related to radioresistance.

many cancer types and are regarded to play a critical role in the development and progression of esophageal cancer and in the promotion of cancer proliferation, invasion, metastasis, and angiogenesis (28). CAFs originate from cells with an activated myofibroblast-like phenotype and are recognized by high levels of fibroblast activation protein-α (FAP) and α-smooth muscle actin (α-SMA). Underwood *et al.* found that most patients with esophageal adenocarcinoma (EAC) express high levels of stromal α-SMA, which predicts a poor survival rate and a poor prognosis. They also observed that α-SMA may increase the invasion potential of esophageal cancer cells through the disruption of the periostin and PI3K-AKT signaling pathways (28). Ji *et al.* indicated that CAFs decrease the radiosensitivity of the lung cancer cell lines A549 and H1299, which significantly contributes to the proliferation and survival of these cancer cells (29).

Tumor-associated macrophages

Tumors have a complex microenvironment that maintains the malignant potential of the tumor and promotes cancer cell invasion and migration; the most abundant cells in the microenvironment are macrophages (30). Macrophages can be divided into two subpopulations of cells. The M1 subpopulation is activated by Toll-like receptor ligands and interferon-γ and plays a role in antitumor immunity, while the M2 subpopulation is activated by interleukin 4 (IL-4) or interleukin 13 (IL-13), each of which suppresses antitumor immunity. Myeloid-derived suppressor cells (MDSCs) are precursors of tumor-associated macrophages and dendritic cells (DCs). Tumor-infiltrating macrophages, which have a predominantly polarized M2 phenotype, play a significant role in the disruption of adaptive immunity; they also contribute to the processes of tumor development and progression (31). M1 macrophages express high levels of

major histocompatibility complex class II in normoxic tumor tissues and antiangiogenic chemokines such as CXCL9 and CXCL10. M2 macrophages express low levels of major histocompatibility complex class II in hypoxic tumor tissues and proangiogenic chemokines such as CCL17, CCL22, and CCL24. Tumor-associated macrophages secrete a large number of growth factors such as PDGF, FGF family members, VEGF, and TGF-β, which play critical proangiogenic roles in esophageal SCC (32). Tumor-associated macrophages also release proteases and matrix proteins such as MMPs, cathepsins and serine proteases to regulate the composition of the ECM and to increase disruption of the basement membrane. Several anti-macrophage approaches, such as the use of a CCL5 receptor antagonist (Met-CCL5), have been evaluated recently; this treatment could downregulate the numbers of tumor-infiltrating macrophages and significantly decreased the tumor volume after radiotherapy in a murine model of esophageal cancer (33). Zoledronic acid and liposomal clodronate reduce the invasion and metastasis of irradiated esophageal SCC through the depletion of tumor-infiltrating $CD11b^+$ monocytes/macrophages that express MMP9.

Regulatory T cells (Tregs)

$CD4^+CD25^+$ Tregs account for approximately 5% of T cells and are recognizable by the expression of FoxP3, which is a transcription factor that is essential for cancer development and progression. Through their suppressive function, Tregs play a critical role in protecting the body against autoimmunity and tissue damage (34,35). Tregs may either be natural Tregs (nTregs) or inducible Tregs (iTregs). nTregs originate in the thymus and mediate suppressive functions through the perforin/granzyme or Fas/Fasl pathways, while inducible Tregs (iTregs) are induced outside the thymus after they are exposed to IL-2, TGF-β and IL-10. Tregs negatively regulate T cell immune responses *in vivo* and promote the invasion, proliferation and metastasis of esophageal cancer (36). Radiotherapy could lead to the formation of a chronic inflammatory microenvironment through modulation of the host immune system, which increases the frequency of Tregs and results in radioresistance and recurrence of malignant tumors. Daclizumab (an anti-CD25 Ab) and the tyrosine kinase inhibitor sunitinib have been used to increase anti-tumor immunity through a reduction in the frequency of Tregs.

Other stromal cells and molecules

Dendritic cells, as potent antigen presenting cells (APCs), present antigens to antigen-specific T cells and mediate the innate and adaptive immune responses (37). Dendritic cells are derived from bone marrow hematopoietic progenitor cells, but they mature within peripheral tissues. Dendritic cells play a dual role in the TAM such as in the mediation of potential anti-tumor immune responses, the activation of cytotoxic T lymphocytes (CTLs) and the blockade of anti-tumor immune responses. Exosomes are multivesicular bodies (MVBs) approximately 30-120 nm in diameter that are derived from luminal membranes; they include abundant bioactive molecules such as miRNA, mRNA, DNA, lipids and proteins (38). Exosomes participate in communication between cells and play a significant role in the balance between development and homeostasis in normal tissues and during oncogenesis. Jelonek *et al.* revealed that exosomes alter their proteins and miRNAs to exert a radioresistant effect (39). Boelens *et al.* found that exosomes derived from the coculture of stromal and breast cancer cells mediate chemoradioresistance through paracrine and juxtacrine signaling (32,40) (*Figure 2*).

Targeted therapy in combination with chemoradiotherapy

The ErbB family includes four tyrosine kinases: ErbB-1 (EGFR), ErbB-2 (HER 2), ErbB-3 (HER 3), and ErbB-4 (HER 4). EGFR is overexpressed in approximately 50-71% of SCC patients and in 9–55% of AC patients (41). EGFR overexpression is associated with a poor prognosis and poor overall survival and is activated by many ligands including EGF, TGF-α and epiregulin. Anti-EGFR monoclonal antibodies (cetuximab and panitumumab) and tyrosine kinase inhibitors (gefitinib and erlotinib) have achieved a significant benefit in clinical trials (32,40,42,43). Safran *et al.* treated 57 patients with esophageal cancer with cetuximab, paclitaxel and RT at a dose of 50.4 Gy/cfx and found that 70% of the patients had a complete clinical response after chemoradiotherapy (44). HER2 is usually identified on the cell surface by immunohistochemistry (IHC) or in the nucleus by fluorescence *in situ* hybridization (FISH) (45). Overexpression of HER2 is common in patients with SCC and in those with AC (approximately 23% and 22%, respectively) and is associated with a poor survival rate (46). The anti-HER2 monoclonal antibody trastuzumab has been demonstrated to improve

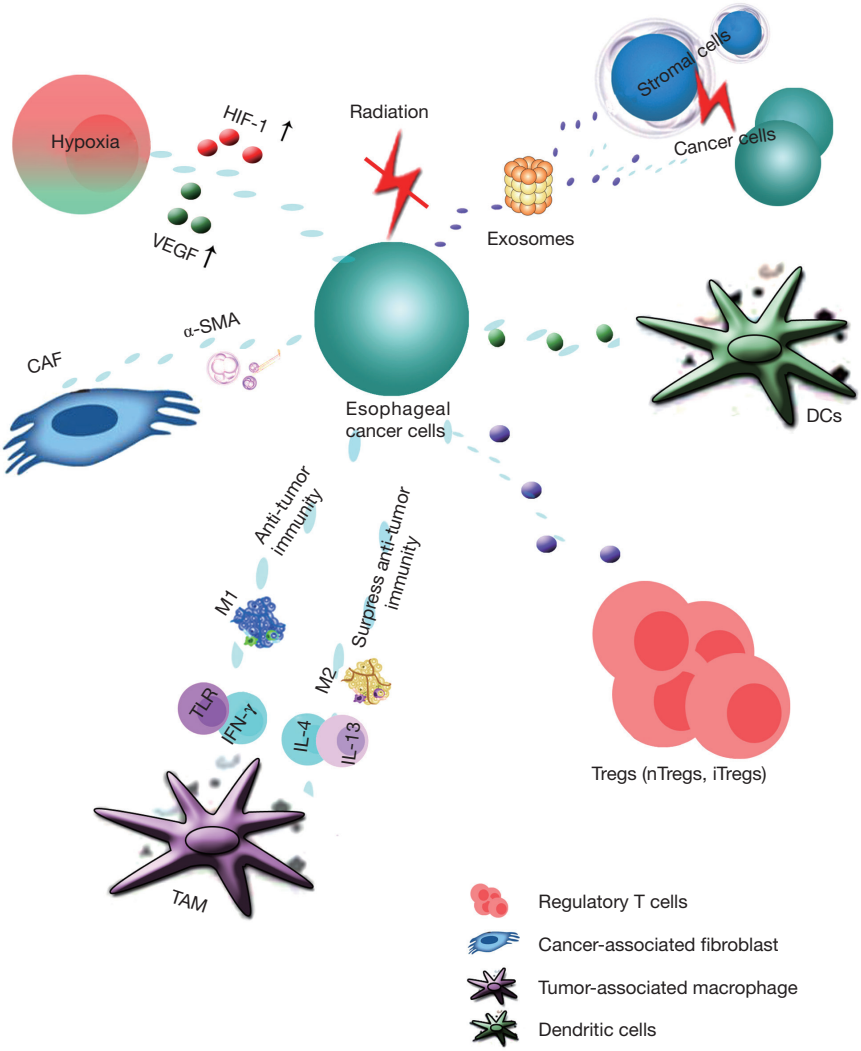

Figure 2 Tumor associated microenvironment (TAM) and radioresistance. Hypoxia can improve the expression of VEGF and HIF-1 and induce radioresistance. Cancer-associated fibroblasts (CAF) play a critical role in the development and progression of esophageal cancer, promoting cancer proliferation, invasion, metastasis, angiogenesis. Tumor-associated macrophages are divided into M1 subpopulation that is activated by Toll-like receptor ligands and interferon-γ, which plays a role in antitumor immunity and M2 subpopulations that is activated by interleukin 4 (IL-4) or interleukin 13 (IL-13), which suppresses antitumor immunity. Tregs, divided into nature Tregs (nTregs) and inducible Tregs (iTregs), play a critical role in protecting itself against autoimmunity and tissue damage through their suppressive function. Dendritic cells play a dual role in tumor-associated microenvironment such as mediating potential anti-tumor immune responses and activate the cytotoxic T lymphocytes (CTLs) or blockade anti-tumor immune responses. Exosomes secreted by stromal cells and esophageal cancer cells mediate radioresistant through paracrine and juxtacrine signaling.

the survival rate of patients with metastatic HER2-positive esophageal cancer, but the effect of the HER2 tyrosine kinase inhibitor lapatinib is still controversial. In a Phase III trial of 584 HER2-positive esophageal cancer patients, trastuzumab was given along with paclitaxel and radiation at a dose of 50.4 Gy/cfx. The median overall survival time was 14.8 *vs.* 11.1 months in the trastuzumab + chemoradiotherapy group and the chemoradiotherapy group, respectively.

VEGF is a critical regulator of both physiologic and pathologic angiogenesis. VEGF can induce endothelial cell mitogenesis, invasion and vascular permeability, and can

mediate tumor growth and metastasis. Overexpression of VEGF is associated with a poor survival rate and advanced cancer stage in 30–60% patients with esophageal cancer; VEGF overexpression also contributes to tumor recurrence and metastasis (47). VEGFR is a predictor of poor prognosis and is overexpressed in 30–50% of esophageal SCC cases. In a retrospective study of 117 esophageal cancer patients conducted by Shih *et al.*, it was demonstrated that the mean number of metastatic lymph nodes was 5.6 *vs.* 3.0 in VEGF-positive cases and VEGF-negative cases, respectively (48). The anti-VEGF monoclonal antibody bevacizumab and the VEGFR tyrosine kinase inhibitor sorafenib have been reported to increase the efficacy of chemoradiotherapy in esophageal cancer patients (49). Meluch *et al.* added bevacizumab, erlotinib, carboplatin and paclitaxel to RT at a dose of 45 Gy (cfx) and treated 62 patients with locally advanced esophageal cancer; the pCR rate was 30% (50).

c-Met is a transmembrane receptor tyrosine kinase, and hepatocyte growth factor (HGF) is the only ligand that binds to this receptor. Aberrant activation of the HGF/Met signaling pathway has been demonstrated to promote the progression and metastasis of esophageal cancer (51). Overexpression of c-Met is associated with an aggressive phenotype and a poor prognosis in patients with esophageal cancer. c-Met promotes motility, proliferation, metastasis and angiogenesis in esophageal SCC through the RAS-MAPK and PI3K-Akt signaling pathways (52). c-Met inhibitors (tivantinib, crizotinib, foretinib) and an HGF inhibitor (rilotumumab) were reported to increase the efficacy of chemoradiotherapy in multiple clinical trials.

Gene expression profiling

Gene expression microarray is a novel high-throughput technology that has been widely used for the identification of the biological characteristics of malignant tumors such as esophageal cancer. Gene expression profile microarrays can analyze thousands of genes and can identify the relevant genes that are related to tumor prognosis (53). In particular, gene expression microarrays have achieved a benefit in terms of their ability to predict responses to neoadjuvant chemoradiotherapy. Maher *et al.* found that five biomarkers (EPB41L3, RTKN, STAT5B, NMES1 and RNPC1) could improve the accuracy in the prediction of the radioresponse of 13 patients with esophageal cancer through DNA microarrays, which were then validated by RT-PCR (54). Duong *et al.* analyzed a group of 46 esophageal cancer patients, which consisted of 21 SCC and 25 AC patients who received neoadjuvant CRT, and found that 32 genes could be used to predict radioresponse by DNA microarray (55). Guo *et al.* revealed that aberrant hypermethylation of RASSF2 is associated with a poor prognosis and that peripheral blood DNA could be used to predict the radioresponse of patients with esophageal cancer (56).

Single nucleotide polymorphisms (SNPs)

As the sequence of the human genome was revealed, we found that genetic variation is larger than previously thought and that the most common variations are SNPs. SNPs have been used to analyze cancer treatment outcome predictor (CTOP) genes and to judge therapeutic effects in esophageal cancer, as most SNPs are silent (57). Nucleotide excision repair genes such as ERCC1 and XRCC1 protect the genome against multiple DNA lesions caused by ionizing radiation. Wu *et al.* investigated variations in SNPs in 210 patients with esophageal cancer using pathway-based approaches and found that the variant allele R399Q in the XRCC1 gene is related to a poor response and could be a prognostic marker in clinical patients (58). Yu *et al.* found that the C118T SNP in the ERCC1 gene could predict response to neoadjuvant radiochemotherapy in 52 patients with esophageal SCC (59).

MicroRNAs

miRNAs are short noncoding RNA sequences 19–24 nucleotides in length that can regulate gene expression through the inhibition of mRNA translation. It has been confirmed that miRNAs are present in tissues and body fluids, where they play a critical role in the progression and recurrence of cancers (60). Odenthal *et al.* analyzed 768 miRNAs using pretherapeutic and post-therapeutic biopsies of 80 esophageal cancer patients and found that miR-192 and miR-194 are significantly related to histopathologic response after neoadjuvant chemoradiotherapy (61). Zhou *et al.* compared miRNA expression in primary ESCCs and recurrent ESCCs after radiotherapy and found that overexpression of miRNA-381 is significantly associated with a decrease in tumor growth and an increase in the radiosensitivity of esophageal cell carcinoma patients (62). Li *et al.* studied 38 patients with ESCC and 19 healthy individuals and found that high levels of plasma miRNA-16 and miRNA-21 are associated with a decrease in progression-free survival (P=0.031 and P=0.038 for miRNA-16 and miRNA-21, respectively) (63).

Proteomics

Proteomics involves the determination of the function of genomic translation and the tumor phenotypes that regulate cancer behavior (64). Proteins are superior biomarkers than other molecules because they influence molecular pathways that are relevant to tumor progression and metastasis (65). Maher *et al.* studied 31 patients with esophageal cancer, 16 of whom exhibited a poor response and 15 of whom exhibited a good response according to the Mandard tumor regression grade (TRG) classification system. They also observed that the serum complement factors C4a and C3a are higher in patients with a poor response and that they predict the response to chemoradiotherapy with sensitivities of 78.6% and 83.3%, respectively (66).

IHC

IHC has an advantage in providing detailed morphological information in a large number of formalin-fixed paraffin-embedded tissue samples and is used widely in the discovery of hypothesis-driven biomarkers. Smit *et al.* investigated esophageal cancer cells that contain a $CD44^+/CD24^-$ subpopulation, which exhibit higher sphere-forming potential and a higher proliferation rate than the $CD44^+/CD24^+$ subpopulation. In a study of preneoadjuvant chemoradiotherapy, in which biopsy material from 27 esophageal cancer patients was examined, the $CD44^+/CD24$ population was identified in 50% of patients with a poor response to chemoradiotherapy. In contrast, this subpopulation was not found in any of the patients who exhibited a complete response, which indicates that the $CD44^+/CD24^-$ population can be a predictive biomarker in esophageal cancer patients in terms of their response to chemoradiotherapy (67).

Medical imaging

Imaging technologies have developed rapidly in recent years. Metabolic and functional imaging modalities such as FDG PET, functional MRI and Hypoxia PET have been used to evaluate the therapeutic effects of radiochemotherapy in patients with esophageal cancer. In their study of 31 patients with esophageal cancer, Klaassen *et al.* found that the hypoxia tracer (^{18}F) HX4 demonstrated good repeatability and may be a potential way to measure treatment response (68). van Rossum *et al.* demonstrated that changes in the apparent diffusion coefficient (ADC) could predict pathologic response to radiotherapy in 20 patients with esophageal cancer through diffusion-weighted magnetic resonance (69).

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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