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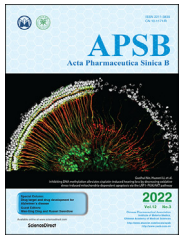

REVIEW

Comparative genomic analysis of esophageal squamous cell carcinoma and adenocarcinoma: New opportunities towards molecularly targeted therapy

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Molecularly targeted therapy

Abstract Esophageal cancer is one of the most lethal cancers worldwide because of its rapid progression and poor prognosis. Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are two major subtypes of esophageal cancer. ESCC predominantly affects African and Asian populations, which is closely related to chronic smoking and alcohol consumption. EAC typically arises in Barrett's esophagus with a predilection for Western countries. While surgical operation and chemoradiotherapy have been applied to combat this deadly cancer, molecularly targeted therapy is still at the early stages. With the development of large-scale next-generation sequencing, various genomic alterations in ESCC and EAC have been revealed and their potential roles in the initiation and progression of esophageal cancer have been studied. Potential therapeutic targets have been identified and novel approaches have been developed to combat esophageal cancer. In this review, we comprehensively analyze the genomic alterations in EAC and ESCC and summarize the potential role of the genetic alterations in the development of esophageal cancer. Progresses in the therapeutics based on the different tissue types and molecular signatures have also been reviewed and discussed.

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1. Introduction

Carcinoma of the esophagus is the seventh most common cancer and the sixth leading cause of cancer-related mortality in the world[1]. Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are two major subtypes of esophageal cancer. ESCC represents the majority of esophageal cancer worldwide, especially in Asian and African populations and is thought to be associated with diet habit and exposure to carcinogens[2]. In contrast, EAC which develops from intestinal metaplasia of the esophageal epithelium due to chronic gastro-esophageal reflux disease is the predominant subtype of esophageal cancer in Western countries[3]. Esophageal cancer is known for its rapid progression and poor outcomes. As esophageal cancer is often asymptomatic in its early stages, the disease is usually at an advanced stage at the time of diagnosis and the prognosis of esophageal cancer is poor with the overall five-year survival rate from 15% to 25%[4]. However, the options for the treatment of esophageal cancer are limited at present. Endoscopic or surgical treatment is applied to early-stage patients, while radiotherapy or/and chemotherapy is predominant for patients with advanced or metastatic cancer[5]. Therefore, it is urgent to develop new therapies targeting the essential factors involving in the initiation and progression of esophageal carcinoma.

Although emerging evidence shows that ESCC and EAC are two different diseases, molecular mechanism of tumorigenesis for each subtype remains unclear. Massively parallel, high-throughput next-generation DNA sequencing (NGS) has enabled comprehensive characterization of somatic mutations and copy number variations (CNVs) in a large scale of samples, which provides new clues and targets for the precise therapy of ESCC and EAC.

In this review, we summarize the recent progress in genomic and molecular characterization of ESCC and EAC. We also propose the opportunities to target the functional alterations for the therapy of ESCC and EAC.

2. Genetic alterations in ESCC and EAC

In light of the molecular events in the tumorigenesis, molecularly targeted therapies have been successfully employed for the treatment of tumors with identified driver genes, including non-small cell lung cancer (*EGFR*, *ALK*), melanoma (*BRAF*), renal cancer (*VEGFR*), hepatocellular cancer (*PDGFRB*), breast cancer (*HER2*, *CDK4*, *CDK6*), chronic lymphocytic leukemia (*BTK*), ovarian cancer (*PARP*) and gastrointestinal cancer (*VEGFR*, *HER2*)[6]. Esophageal cancer has been benefitted little from the targeted therapies due to the unclear pathogenesis. Therefore, defining genomic landscape of esophageal cancer would gain insights into the initiation and development of esophageal cancer and facilitate to develop molecularly targeted therapies. NGS has revolutionized cancer genomic research by providing a comprehensive profiling of cancer genome, including point mutations, insertions, deletions and copy number variations, making it a powerful tool to identify potential driver genes or pathways of human cancer[7]. In recent years, several NGS studies have been performed in esophageal cancer patients across the world. The cBioPortal for cancer genomics (https://www.cbioportal.org) is an open platform integrating cancer genomics data from multiple resources with high credibility. We obtained the genomic data of ESCC and EAC samples from cBioPortal (Supporting Information Tables S1−S4) and compared their genomic profile in an effort to identify potential therapeutic targets. The top 15 cancer-related genes with highest rate in mutation or copy number variation in ESCC or EAC were presented in Fig. 1. The genes in red have been reported to be associated with esophageal cancer and are or potentially are therapeutic targets for esophageal cancer. As shown in Fig. 1, *TP53*, *PIK3CA* and *KMT2D* are commonly mutated (Fig. 1A), and copy number variations in *CCND1*, *FGF3*, *FGF4*, *FGF19*, *CDKN2A* and *CDKN2B* are frequently found (Fig. 1B) in both subtypes of esophageal cancer, while distinct genetic alterations were also found in ESCC and EAC. Here, we summarize these cancer-related genes and outlined their potential functions in ESCC and EAC.

2.1. *Genetic alterations with similar frequency in ESCC and EAC*

By comparing the frequency of genetic alterations between ESCC and EAC, we found that *TP53*, *CDKN2A*, *MYC* and *KMT2C* are commonly altered with similar frequency in both subtypes of esophageal cancer (Fig. 2), reflecting deregulation in genomic stability and cell cycle progression in both ESCC and EAC (Fig. 3).

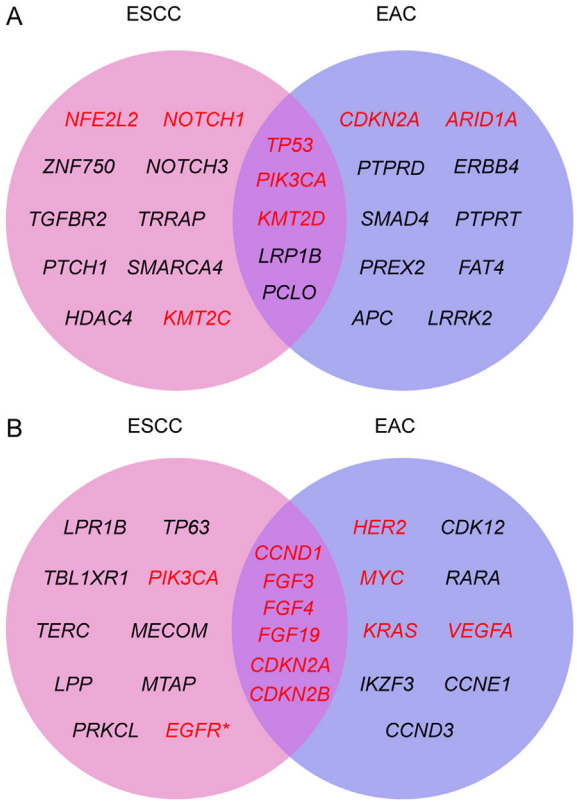

Figure 1 Frequent genetic alterations in human esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). The data of genetic alterations in human ESCC and EAC were collected from cBioPortal, www.cbioportal.org, accessed in April 2021 (Tables S1−S4). The top 15 cancer-related genes with highest rate in mutation (A) or copy number variation (B) in ESCC and EAC were presented as a Venn diagram. Genes in red have been reported to be associated with esophageal cancer and are or potentially are therapeutic targets. **EGFR* has been verified as a promising target to treat ESCC though it is not among the top 15 altered genes.

2.1.1. *TP53*

The *TP53* gene, localizing on chromosome 17p13.1, encodes a tumor suppressor protein responding to diverse cellular stresses to ensure cell and tissue homeostasis[8]. Somatic mutations of *TP53* are one of the most common alterations in human cancer, and about 80% of esophageal cancer patients carry *TP53* mutations (Figs. 2A and 3). Most of them are hotspot mutations in DNA-binding domain[9] (codons 175, 245, 248, 273, and 282), and truncated mutation at R342X in P53 tetramerization motif. These mutations abrogate the tumor suppressive function of P53, resulting in unstable genome. A recent study indicated that G245C and R273H mutants played important roles in tumorigenesis of ESCC, which might be exploited for diagnosis and therapy of patients carrying the mutants[10]. In a multivariable analysis in 161 patients with resectable ESCC, researchers revealed that the non-disruptive mutation in *TP53* DNA-binding domain is a potential independent prognostic factor indicating prolonged survival and patients with elevated P53 expression showed a better outcome[11]. A genomic analysis of Barrett's esophagus (BE) patients found significantly higher numbers of *TP53* mutations in BE patients with progression to EAC[12]. Taken together, these results suggest *TP53* mutations are closely linked to the pathogenesis of esophageal cancer, which could be used as potential prognosis biomarker dependent on disease contexts.

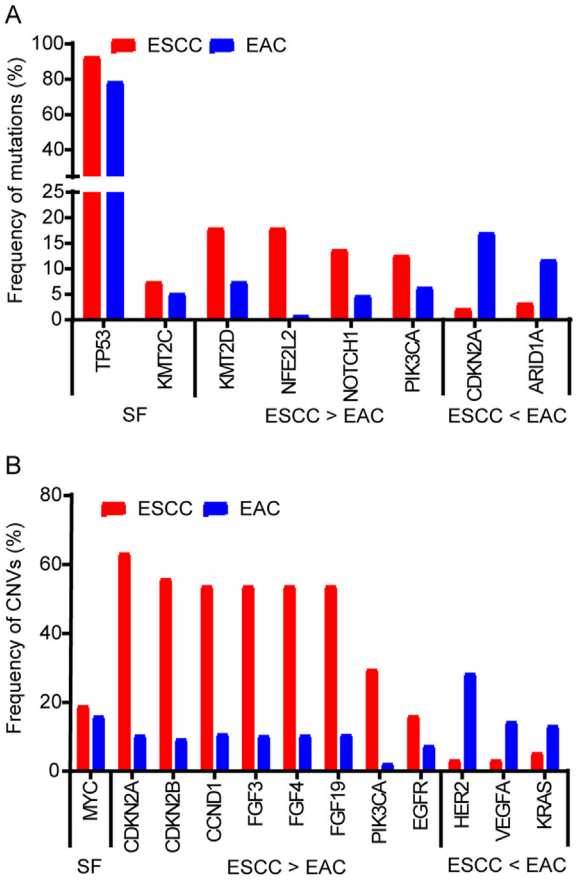

Figure 2 Comparison of genetic alterations between ESCC and EAC. The frequency of alteration of the genes in red presented in Fig. 1 was compared between ESCC and EAC according to the data from cBioPortal. SF: similar frequency. If the frequency of alteration in a gene is greater than 2-fold (≥2) in ESCC compared to that in EAC, the gene is assigned to 'ESCC > EAC'. If the frequency of alteration in a gene is greater than 2-fold (≥2) in EAC compared to that in ESCC, the gene is assigned to 'ESCC < EAC'. If the difference in the frequency of alteration in a gene is less than 2-fold, the gene was considered as 'similar frequency' in both ESCC and EAC. (A) Frequency of mutations. (B) Frequency of copy number variations.

2.1.2. *CDKN2A*

CDKN2A encodes a cyclin-dependent kinase inhibitor p16 (Ink4a), a tumor suppressor that regulates the transition of cell cycle from G1 to S phase. The *CDKN2A* gene is located within the frequently deleted region of chromosomal 9[13]. Loss of p16 is common in variant cancers. Inactive or decreased p16 fails to bind to cyclin D, which assists cyclin-dependent kinase 4/6 (CDK4/6) in phosphorylating Rb protein, and results in loss of brake in cell cycle progression. *CDKN2A* deletion is found in about 63% ESCC (Figs. 2B and 3A). A comprehensive analysis of whole-genome sequencing (WGS) of 31 ESCC tumors and paired normal samples found that *CDKN2A* was deleted in 29 out of 31 ESCCs[14]. Homozygous deletion and promotor methylation of *CDKN2A* were also found to occur in 86% of a cohort of 21 cases of ESCC[15]. In addition, loss-of-function mutations of *CDKN2A* are detected in about 17% EAC patients (Figs. 2A and 3B). A whole exome sequencing from 25 pairs of EAC and BE revealed that the progression of BE often emerged with the inactivation of *CDKN2A*[16]. Furthermore, researchers have verified that methylation of the *CDKN2A* promotor is the predominant mechanism of p16 inactivation, which is a very common event in EAC and occurs as early as metaplasia[17].

2.1.3. *MYC*

The *MYC* gene, located on chromosome 8q24.21, encodes a nuclear phosphoprotein that plays important roles in cell cycle progression, apoptosis and cellular transformation. The amplification of *MYC* occurs frequently in a variety of human cancers, contributing to tumor progression and indicating poor outcome[18]. According to the data from cBioPortal, *MYC* amplification occurs in nearly 20% esophageal cancer patients (Figs. 2B and 3). *MYC* amplification was detected in the normal mucosa adjacent to the tumor in 23% of a group of esophageal cancer patients from high-risk region of China[19]. High expression level of MYC was significantly correlated with poor prognosis and also associated with invasion and lymph node metastasis in ESCC patients[20]. The amplification of *MYC* is also frequently detected in EAC, which is likely to be an early event during EAC carcinogenesis[21].

2.1.4. *KMT2C*

KMT2C, mapped on chromosome 7q36.1, also known as myeloid/lymphoid or mixed-lineage leukemia protein 3 (MLL3), encodes a nuclear protein which possesses histone methylation activity and participates in transcriptional coactivation. The lysine methyltransferase 2 (KMT2) family members play essential roles in regulating cell growth and development, and mutations in these genes are frequently found in blood and solid cancers[22]. *KMT2C* has been reported to be frequently mutated in a variety of tumors. There are about 5% esophageal cancer patients carrying mutations in *KMT2C* (Figs. 2A and 3), which have also been identified in other studies[23,24]. However, the function of *KMT2C* in esophageal cancer and the relationship between *KMT2C* mutations and clinicopathological characteristics of esophageal cancer remain poorly understood.

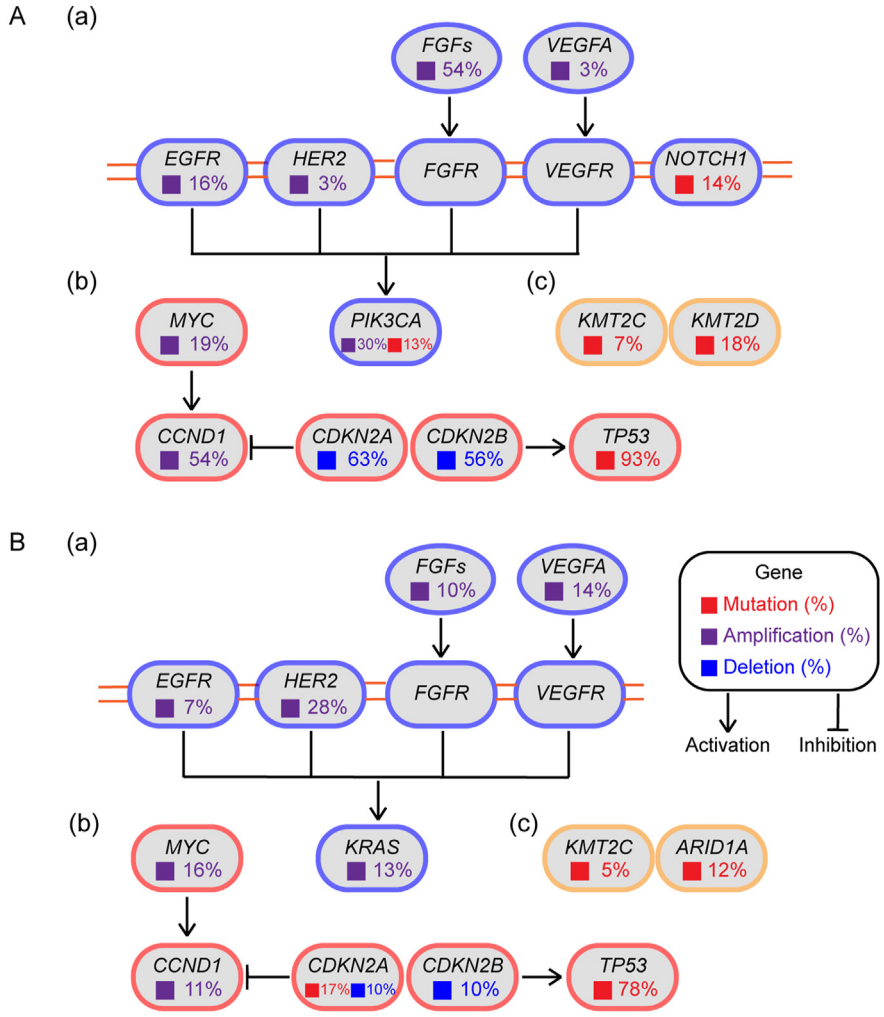

Figure 3 Significantly dysregulated pathways in human ESCC (A) and EAC (B). The most altered genes were classified into three signaling pathways. The frequency of genetic alterations in ESCC and EAC were retrieved from cBioPortal (www.cbioportal.org, accessed in April 2021.). (a) RTK−MAPK−PI3K signaling. (b) Cell cycle regulation. (c) Epigenetic modulation.

2.2. *Differential genetic alterations in ESCC and EAC*

Though ESCC and EAC share a few common genetic alterations, they exhibit distinct molecular profile as two subtypes of esophageal cancer (Fig. 2). In terms of genetic mutations, significantly higher mutational rates in *NFE2L2*, *KMT2D*, *NOTCH1* and *PIK3CA* are found in ESCC, whereas more frequent mutations in *CDKN2A* and *ARID1A* are observed in EAC (Fig. 2A). There are also distinctions in CNVs between ESCC and EAC. *HER2*, *VEGFA* and *KRAS* are significantly more amplified in EAC, while ESCC exhibits higher CNVs of *CDKN2A*, *CDKN2B*, *CCND1*, *FGFs*, *PIK3CA* and *EGFR* (Fig. 2B). These molecular differences suggest ESCC and EAC should not be considered as one disease and the therapeutic targets might be different.

2.2.1. *Genetic alterations with higher frequency in ESCC*

2.2.1.1. FGFs The family of fibroblast growth factors (FGFs) regulates a wide range of biological functions, including cellular proliferation, survival, migration and differentiation[25]. There is mounting evidence for the importance of FGF signaling in the pathogenesis of various tumor types, and clinical inhibitors targeting FGFs or their receptors are being rapidly developed[26]. *FGF3/4/19*, located at 11q13-14, are found amplified in more than 50% ESCC patients (Figs. 2B and 3A), which often coincided with *CCND1* amplification. Amplification of *FGF3/4* was detected by DNA microarrays in 9 of 20 surgically resected primary ESCC tumors[27]. In a cohort of 3342 gastroesophageal cancers, amplification of *FGF3*, *FGF4*, *FGF19* and *FGFR1* were significantly more frequent in ESCC compared with EAC[28], which indicated the preferential activation of FGF pathway in ESCC. Fibroblast growth factor receptor 1 (FGFR1), FGFR4 and fibroblast growth factor receptor like 1 (FGFRL1) was implicated in the progression of ESCC[29], and genetic depletion of *FGFRL1* was able to attenuate the motility and invasion in ESCC cells[30]. Moreover, several studies have demonstrated that *FGFR1* amplification was an independent factor of poor prognosis in ESCC patients[31,32]. Given that the defined roles of abnormal FGF/FGFR signaling in tumorigenesis, agents targeting FGF/FGFR may be a potential opportunity for ESCC treatment.

2.2.1.2. EGFR *EGFR*, located on chromosome 7p11.2, encodes a transmembrane glycoprotein that belongs to the ERBB family of receptor tyrosine kinases (RTK). Binding of epidermal growth factor receptor (EGFR) to epidermal growth factors

triggers its autophosphorylation and subsequent activation of signal transduction pathways leading to cell proliferation, differentiation and survival[33]. Abnormal activation of EGFR is often found in epithelial tumors and related to poor prognosis and decreased survival[34]. Amplification of *EGFR* occurs in about 16% ESCC patients (Figs. 2B and 3A), which was significantly associated with overexpression of EGFR[35]. The survival rate of patients with high EGFR expression was significantly lower than that of patients with lower expression in a study consisting of 441 ESCC patients, suggesting that expression of EGFR could be a prognostic predictor for ESCC[36]. Furthermore, a molecular prognostic model have showcased that comprised expression of EGFR, p-Sp1 and Fascin proteins was significantly associated with poor clinical outcome of ESCC patients[37].

2.2.1.3. NOTCH1 The *NOTCH1* gene, located on chromosome 9q34.3, encodes a member of the NOTCH family proteins. NOTCH signaling is an evolutionarily conserved intercellular signaling pathway that regulates interactions between physically adjacent cells through binding of NOTCH family receptors to their cognate ligands. There is emerging evidence that mutations in *NOTCH* genes and dysregulated NOTCH signaling are important factors in tumor initiation and development[38–40]. *NOTCH1* mutation is found in 14% ESCC according to the data from cBioPortal (Figs. 2A and 3A). The mutations of *NOTCH1* in ESCC tended to cluster in the EGF-like repeats and resulted in loss of function[41]. Loss of NOTCH1 was found to predispose the esophagus to precancer and squamous cell carcinoma partially *via* accelerated telomere erosion[42]. Constitutively active NOTCH signaling pathway, regardless of transient or stable expressions of NOTCH1, was able to inhibit the proliferation of ESCC EC9706 cells *via* arresting cells at G1 phase[43]. Analysis of NGS data from 104 ESCCs from China found that patients with loss of function *NOTCH1* mutations failed to respond to chemotherapy and had shorter survival times than patients without mutations in this gene[44]. Therefore, NOTCH seems to act as a tumor suppressor in the development of ESCC.

2.2.1.4. PIK3CA The *PIK3CA* gene, mapped on chromosome 3q26.32, encodes the catalytic subunit of phosphoinositide 3-kinase (PI3K), p110α. Class I PI3K is a lipid kinase composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit, which is responsible for phosphorylating phosphatidylinositol-4,5-bisphosphate (PIP_2) to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3). The PI3K signaling pathway regulates various cellular processes, such as proliferation, cell cycle, apoptosis and cytoskeletal rearrangement[45]. PI3K pathway is frequently disturbed in many human cancers, mostly *PIK3CA* alterations[46]. The mutation and amplification of *PIK3CA* occur in 13% and 30% ESCC patients respectively (Figs. 2 and 3A), which have been verified in a number of studies[23,24,47]. A comprehensive analysis of PI3Kα expression in ESCC patients indicated that overexpression of PI3Kα was associated with lymph node metastasis and PI3Kα may play a crucial role in the development of ESCC[48]. *PIK3CA* amplification was also associated with shorter survival in an investigation of 534 curatively resected ESCC patients, suggesting its role as a poor prognostic factor in resected ESCC[49]. The alteration of *PIK3CA* is common in ESCC, making it a promising target for ESCC treatment and potential biomarkers for individualized molecularly targeted therapy for ESCC[50].

2.2.1.5. CDKN2B The gene of *CDKN2B* encodes a cyclin-dependent kinase inhibitor p15, which interacts with CDK4 or CDK6, and prevents the activation of CDKs. Thus, the function of p15 is to regulate cell cycle progression by restraining G1/S transition. *CDKN2B* is located on chromosome 9p21.3, a region often implicated in the pathogenesis of multiple cancers. Genetic variants at 9p21.3 may modulate the expression of p15 and contribute to ESCC susceptibility[51,52], highlighting the importance of 9p21.3 genetic variants in tumorigenesis. Among the genetic variants found in this region, deletion of *CDKN2B* is the most common alteration in ESCC, which occurs in 56% ESCC patients (Figs. 2B and 3A). In a genetic analysis of 33 human esophageal carcinomas and their adjacent normal tissues, deletion of *CDKN2B* was observed in a large proportion of ESCC samples[53]. Additionally, the homozygous deletion of *CDKN2B* was notably associated with lymph node metastasis in ESCC[54]. Lately, a study revealed *CDKN2A/2B* loss could be a biomarker predictive of CDK4/6 inhibitor efficacy in both patient-derived cell and patient-derived xenograft models[55], which is consistent with disorder of cell cycle in ESCC due to *CDKN2A/2B* deletion.

2.2.1.6. CCND1 *CCND1*, located on chromosome 11q13.3, encodes a highly conserved cyclin family member cyclin D1, which forms a complex with CDK4/6 and participates in cell cycle G1/S transition. Mutation, amplification and overexpression of this gene are able to release a cell from its controlled cell cycle and cause transformation to a malignant phenotype[56]. The amplification of *CCND1* is detected in 54% ESCC patients (Figs. 2B and 3A). In a whole exome sequencing analysis of tissues collected from 144 Japanese ESCC patients, cell cycle regulators constituted the most frequently disrupted category, including *TP53* mutations (93.1%), *CCND1* amplification (46.5%) and *CDKN2A* deletion (53.5%)[57]. Another study with 55 ESCC patients also found that ratios of *CCND1* amplification or cyclin D1 overexpression were 42% or 58%, respectively[58]. High expression of cyclin D1 was able to increase distant metastasis, decrease overall survival and distant metastasis-free survival in resectable ESCC, which may help to identify patients with high risk of postoperative metastases[59]. Collectively, the alterations of *CCND1* exist in almost half of ESCC patients, manifesting its critical character in the development and progression of ESCC.

2.2.1.7. KMT2D *KMT2D*, localized to chromosome 12q13.12, encodes a histone methyltransferase that is responsible for methylating histone 3 at lysine 4. *KMT2D* plays critical roles in regulation of development, differentiation, metabolism and tumor suppression[60]. Frequent mutations in genes involving in histone modification including *KMT2D* have been found in ESCC. *KMT2D* mutations occur in 18% of ESCC (Figs. 2A and 3A), the majority of which are truncated mutations resulting in proteins lacking the key methyltransferase domain, indicating a tumor suppressor role of *KMT2D* in ESCC[23,24,28,47,57]. Notably, *KMT2D* was mutated in 60% of the metastatic ESCC and in only 15.3% of the primary ESCC, proposing an important role of *KMT2D* in ESCC metastasis[61]. However, the clinical significance and

prognostic value of *KMT2D* mutations in ESCC have not been fully elucidated, which deserves further investigation.

2.2.1.8. NFE2L2 The *NFE2L2* gene, mapped on chromosome 2q31.3, encodes a transcription factor which is a member of small family of basic leucine zipper proteins. NFE2L2 is the master regulator of cellular antioxidant responses. Mutations of *NFE2L2* frequently occur in human cancers, which drive continuous NFE2L2 activation and correlate with poor prognosis[62]. *NFE2L2* mutations is found in 18% ESCC according to the data from cBioPortal (Fig. 2A). An analysis of 1145 tumor samples detected that *NFE2L2* mutations existed in 11.4% samples, accompanied with increased NFE2L2 expression in the nuclei[63]. Recently, a WGS of 508 Chinese ESCC patients identified that *NFE2L2* mutations were significantly associated with worse prognosis of ESCC[64]. Mutant NFE2L2 conferred attachment-independent cell survival, which was correlated with lymph node metastasis and tumor progression[65]. Down-regulation of mutant NFE2L2 by short hairpin RNA increased the sensitivity of ESCC cells to chemotherapy[65]. These results indicate the oncogenic function of *NFE2L2* mutation in ESCC and strategies are proposed to target the NFE2L2 for future therapy[66].

2.2.2. Genetic alterations with higher frequency in EAC

2.2.2.1. HER2 *HER2*, located on chromosome 17q12, encodes a member of EGFR family of RTKs. Although HER2 possesses an active tyrosine kinase domain, no direct ligand has been identified. HER2 exists in the extended active conformation, rendering HER2 constitutively available for dimerization[67,68]. *HER2* is often amplified in human cancers such as breast cancer, gastric and esophageal cancer[69–71]. According to the data from cBioPortal, *HER2* amplification occurs in 28% of EAC (Figs. 2B and 3B). The HER2 expression in EAC patients is highly concordant with its copy number[72]. The overexpression of HER2 was also a typical feature in an animal model of BE-related EAC[73], indicating a crucial role of HER2 in EAC pathogenesis. HER2 expression is likely to indicate a positive prognosis in EAC. Several studies have reported that EAC patients with high HER2 expression showed a superior overall survival compared to HER2-negative patients[72,74,75]. In consideration of the vital role in EAC, HER2 has become a therapeutic target for EAC patients.

2.2.2.2. VEGFA *VEGFA*, located on chromosome 6p21.1, encodes a heparin-binding protein, which exists as a disulfide-linked homodimer. This growth factor stimulated proliferation and migration of vascular endothelial cells, which is essential for angiogenesis under both physiological and pathological circumstances. As angiogenesis is important to provide nutrients for tumor growth, vascular endothelial growth factor A (VEGFA) is highly expressed in cancer tissues and associated with more aggressive features. Data from cBioPortal shows that *VEGFA* amplification occurs in 14% of EAC (Figs. 2B and 3B). In consistency with the high frequency of amplification, significant increase in VEGFA expression was found in tumor and peritumoral adipose tissue during the progression of normal squamous epithelium to EAC[76,77]. Elevated expression of VEGFA was also observed in lymph node metastases of EAC, indicating an important role in tumor metastasis[78]. Furthermore, VEGFA expression was significantly associated with poor prognosis, which supports the targeted-VEGFR therapy for EAC patients[79].

2.2.2.3. KRAS The *KRAS* gene located on chromosome 12p12.1 encodes the small GTPase KRAS. KRAS acts as a molecular switch, regulating diverse signal transduction pathways[80]. As one of the most well-known proto-oncogenes, *KRAS* has been intensively studied in the past years. The activating mutations of *KRAS* occur in approximately 30% of all human cancers, indicating its pivotal role in driving cancer progression[81]. Nevertheless, the amplification of *KRAS* is more common in EAC. In a WGS analysis of 22 fresh-frozen EAC samples, the amplification of *KRAS* by breakage-fusion-bridge was found in 6 samples, proposing a potential role in fueling EAC progression through *KRAS* amplification[82]. Additionally, *KRAS* amplification significantly related with nodal positive patients and poorer survival in the subgroup without neoadjuvant treatment. Co-existing of amplification in *KRAS*, *HER2* as well as *TP53* mutations has been reported to indicate an unfavorable prognosis in EAC[83].

2.2.2.4. ARID1A *ARID1A*, mapped on chromosome 1p36.11, encodes a member of SWI/SNF family, which is thought to regulate transcription of specific genes by modulating the chromatin structure. Inactivating mutations in *ARID1A* have been identified in a broad spectrum of cancers, indicating its action as a tumor suppressor[84]. There are about 12% of EAC harboring *ARID1A* mutations (Figs. 2A and 3B). In a WGS study of endoscopic biopsies, *ARID1A* mutations were detected in 15% of high-grade dysplasia or EAC and loss of ARID1A was also identified in BE/high-grade dysplasia/EAC tissues. ARID1A-deficient EAC was reported to be accompanied with high microsatellite instability[85], suggesting its role in genomic stability. Knock down of ARID1A in EAC OE33 cells was able to enhance cell growth, proliferation and invasion, suggesting its suppressive activity in EAC pathogenesis[86]. The overall survival time of patients with loss of *ARID1A* is apparently shorter than those with wild-type *ARID1A* (26.2 months *vs*. 60.1 months)[87]. Collectively, *ARID1A* acts as a tumor suppressor in the initiation and progression of EAC.

3. Emerging therapeutic targets in esophageal cancer

With the development of molecularly targeted drugs and revelation of genetic alterations in esophageal cancer, molecularly targeted therapy has emerged as a promising opportunity for the treatment of esophageal cancer. Here, we summarized the recent progress in the treatment of ESCC and EAC by exploiting their distinct genetic alterations.

3.1. Common therapeutic targets in esophageal cancer

3.1.1. c-MET

c-MET is a transmembrane receptor tyrosine kinase, which is activated by hepatocyte growth factor (HGF) *via* autophosphorylation at the activation loop of the kinase domain. The signal mediated by c-MET is involved in cell proliferation, motility, invasion and survival. The amplification and overexpression of c-MET is implicated in a wide variety of human cancers including esophageal cancer[88,89]. *MET* amplification was an independent

prognosis factor in surgical ESCC and negatively affected the survival of ESCC patients[90]. The esophagogastric adenocarcinoma patients with *MET* amplification showed a substantially shorter median survival time and more sensitive to the c-MET inhibitor crizotinib[91]. These findings suggest c-MET may be a potential therapeutic target for esophageal cancer and crizotinib is being tested in clinical trials (Fig. 4 and Table 1). Anti-c-MET antibody telisotuzumab showed acceptable safety profile and clinical activity in esophageal cancer patients carrying *MET* amplification. Among four gastroesophageal cancer patients, three achieved a partial response and one had progressive disease as best response[92]. The completed phase I and II clinical studies with c-MET inhibitor AMG337 found that EAC patients with *MET* amplification responded well to AMG337 with manageable toxicities, and an objective response rate of 18% was observed in gastric/gastroesophageal junction/EAC patients with *MET* amplification[93,94]. Collectively, c-MET is a promising target for esophageal cancer patients with *MET* amplification.

3.1.2. *CDK4/6*

The cell cycle-related genes including *TP53*, *CDKN2A*, *CDKN2B* and *CCND1* frequently altered in esophageal cancer (Fig. 3), indicating a highly disordered cell cycle progression. In recent years, therapies targeting cell cycle progression has made great progress. Three CDK4/6 inhibitors, abemaciclib, palbociclib and ribociclib, have emerged for the therapy of breast cancer. In light of the dysregulation of cell cycle, targeting CDK4/6 might be a promising approach for treatment of esophageal cancer. Abemaciclib was able to induce apoptosis and inhibit proliferation in EAC cells and significantly inhibited the tumor growth *in vivo*[95]. Clinical trials to test the efficacy of CDK4/6 inhibitors in esophageal cancer are warranted.

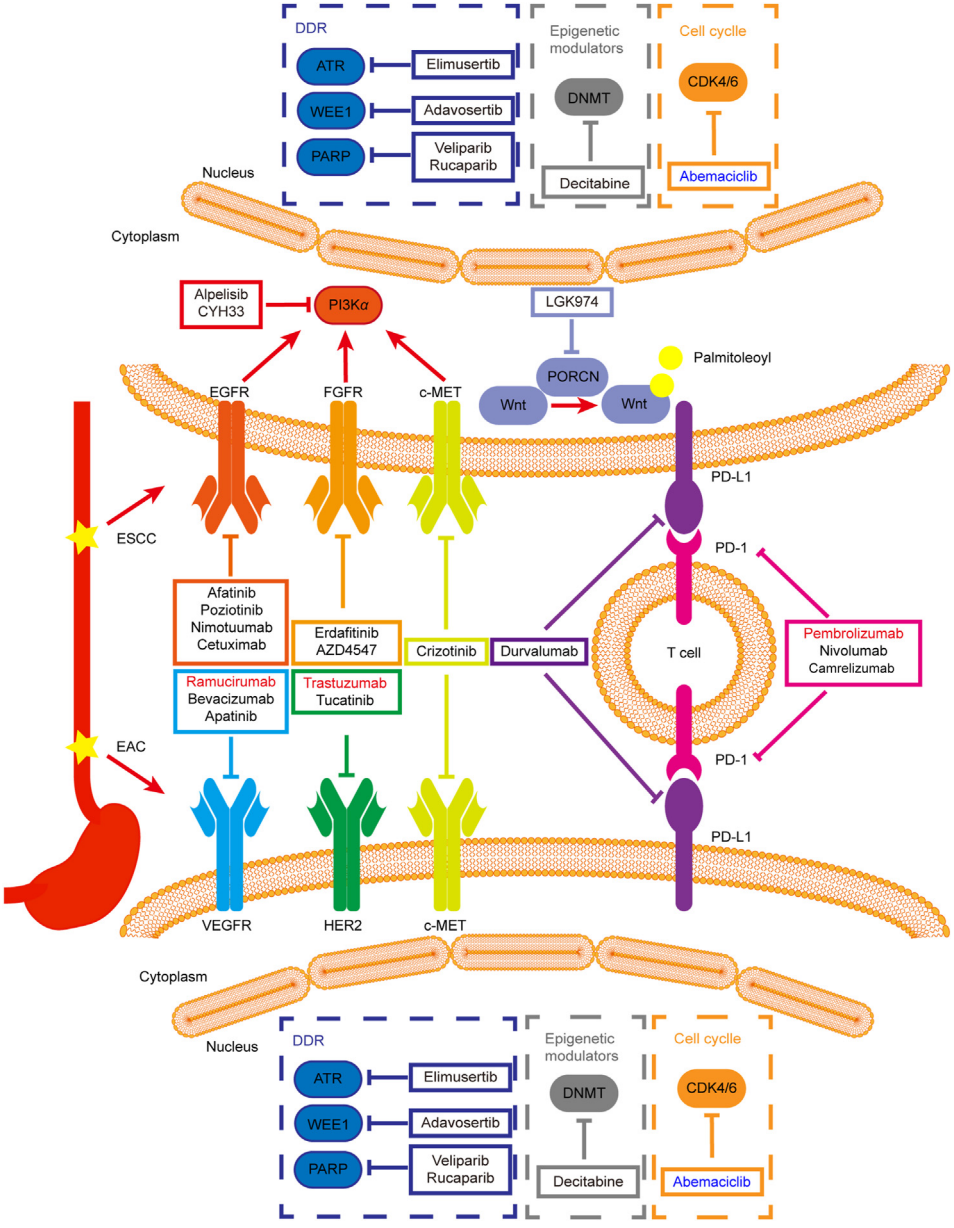

Figure 4 Potential therapeutic targets and drugs for the treatment of ESCC and EAC. Drugs in red have been approved by FDA, drugs in black are at different stages of clinical trials, while drugs in blue are in preclinical studies.

Table 1 Ongoing clinical trials of molecularly targeted drugs in esophageal cancer.

NCT No.	Drug	Target	Disease	Phase
NCT02465060	Crizotinib	c-MET	Esophageal cancer	II
NCT04491942	Elimusertib + chemotherapy	ATR	Esophageal cancer	I
NCT04460937	Adavosertib + radiotherapy	WEE1	Esophageal cancer	I
NCT04171700	Rucaparib	PARP	Esophageal cancer	II
NCT01366144	Veliparib + chemotherapy	PARP	Esophageal cancer	I
NCT03233724	Decitabine	DNMT	Esophageal cancer	I/II
NCT03544736	Nivolumab + radiotherapy	PD-1	Esophageal cancer	I/II
NCT03691090	Camrelizumab + chemotherapy	PD-1	Esophageal cancer	III
NCT02639065	Durvalumab	PD-L1	Esophageal cancer	II
NCT03940976	Afatinib	EGFR	ESCC	II
NCT02409186	Nimotuzumab + chemoradiotherapy	EGFR	ESCC	III
NCT03770988	Poziotinib	EGFR	ESCC	II
NCT03126708	Cetuximab	EGFR	ESCC	II
NCT02699606	Erdafitinib	FGFR	Esophageal cancer	II
NCT01795768	AZD4547	FGFR	Esophageal cancer	II
NCT03292250	Alpelisib	PI3Kα	ESCC	II
NCT03544905	CYH33	PI3Kα	ESCC	I
NCT01351103	LGK974	Wnt	ESCC	I
NCT04430738	Tucatinib	HER2	EAC	I/II
NCT03783936	Trastuzumab + avelumab	HER2	EAC	II
NCT04499924	Tucatinib	HER2	EAC	II/III
NCT01359397	Bevacizumab + chemotherapy	VEGFR	EAC	II
NCT02898077	Apatinib + chemotherapy	VEGFR	Esophageal cancer	II

3.1.3. *Genes involving DNA damage response*

Esophageal cancer is characterized by a high mutation in *TP53*, which plays pivotal roles in the DNA damage response[96]. In response to DNA damages, activation of P53 initiates pathways involved in cell cycle arrest, DNA repair, and apoptosis in order to avoid propagation of damaged cells. Mutations in *TP53* cause defective and weakened G1 and G2 checkpoints, rendering cells highly dependent on activated WEE1 to achieve cell cycle arrest in response to DNA damage. WEE1 was identified as a synthetic lethal target in *TP53*-mutated cancer. WEE1 inhibitor AZD1775 in combination with chemotherapy showed superior response rate in *TP53* mutated patients (21%) compared with those with wild-type *TP53* (12%)[97]. Combination DNA damage response inhibitors such as inhibitors targeting ATR, CHK1/2 with chemotherapy/radiotherapy also displayed potential for the treatment of esophageal cancer[98]. The combination of an ATR inhibitor, elimusertib, or a WEE1 inhibitor, adavosertib, with chemotherapy/radiotherapy for the treatment of advanced solid tumors including esophageal carcinoma is being tested in clinical trials (Fig. 4 and Table 1). PARP inhibitors alone or in combination with paclitaxel are being evaluated in esophageal cancer patients carrying deleterious mutation in homologous recombination repair genes (Fig. 4 and Table 1).

3.1.4. *Epigenetic modulators*

Epigenetic alterations such as *KMT2C*, *KMT2D* and *ARID1A* are common in esophageal cancer (Fig. 3). Though the role of these alterations has not been clearly elucidated, drug candidates targeting epigenetic modulators have displayed potential activity against esophageal cancer. Pretreatment of the DNA methyltransferase inhibitor azacitidine enhanced the efficacy of chemotherapy in advanced EAC patients. All 12 patients achieved R0 section after treatment of azacitidine plus chemotherapy. 3 patients (25%) had a complete response, 5 patients (42%) had a partial response, and 4 (33%) had stable disease[99]. Combination of a histone deacetylase inhibitor MS-275 and azacytidine was efficient to inhibit esophageal cancer cells, indicating a potential targeted therapy for the esophageal cancer[100]. Moreover, the DNA methyltransferase (DNMT) inhibitor decitabine is being tested in clinical trials (Fig. 4 and Table 1). As the role of epigenetic alterations in esophageal cancer is gradually revealed, epigenetic-targeted therapy might be a possible option for esophageal cancer patients.

3.1.5. *Immune checkpoint*

For the past few years, remarkable breakthrough of immunotherapy has been made for the treatment of solid tumors. Immune checkpoint inhibitors such as anti-CTLA4 or anti-PD-1/PD-L1 antibodies have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of multiple solid tumors. PD-1 inhibitor pembrolizumab exhibits desired activity in esophageal cancer patients, which has been used to treat ESCC with high expression of PD-L1 (Fig. 4 and Table 2)[101]. Immune checkpoint inhibitors alone or combined with chemoradiotherapy are being assessed in clinical trials (Fig. 4 and Table 1) and encouraging results have been obtained in esophageal cancer. In a single-arm phase II trial, PD-1 inhibitor camrelizumab combined with apatinib and chemotherapy demonstrated significant efficacy and manageable safety in advanced ESCC patients. The objective response rate and disease control rate were 80% and 96.7%

Table 2 Approved molecularly targeted drugs for esophageal cancer.

Drug	Target	Company	Indication
Trastuzumab	HER2	Pfizer	EAC
Ramucirumab	VEGFR	Lilly	EAC
Pembrolizumab	PD-1	Merck Sharp & Dohme	ESCC

respectively. All adverse events were handled with appropriate medical care, which suggested a new combination strategy for ESCC treatment[102]. The level of serum lactate dehydrogenase may serve as a potential marker for the response of camrelizumab in ESCC patients[103]. Recently, the efficacy and safety of pembrolizumab in combination with chemoradiotherapy are being evaluated in both ESCC and EAC patients, which may help define the role of immunotherapy as a first-line treatment option for patients with esophageal carcinoma[104].

3.2. *Therapeutic targets in ESCC*

3.2.1. *EGFR*

Amplification and overexpression of EGFR occurred frequently in ESCC and significantly correlated with tumor progression and invasion[35,58,105], which indicates that EGFR is a potential molecular target in ESCC. The selective EGFR inhibitor gefitinib showed potent antitumor activity against ESCC *in vitro* and *in vivo* and augmented the efficacy of tumor necrosis factor-related death apoptosis inducing ligand in human ESCC cells[106,107]. Lapatinib, a dual inhibitor of EGFR and HER2, possessed significant activity in ESCC cells over-expressing EGFR and/or HER2 and synergistically inhibited the growth of ESCC patient-derived xenograft in combination with 5-fluorouracil[108,109]. Monoclonal antibodies against EGFR also exhibited favorable efficacy against ESCC. Nimotuzumab was able to promote radiosensitivity in EGFR-overexpressing ESCC cells by upregulating insulin like growth factor binding protein 3 (IGFBP-3)[110]. Cetuximab significantly inhibited the growth of ESCC cells overexpressing EGFR[111]. Clinical trials of small molecule inhibitors or monoclonal antibodies targeting EGFR for the treatment of ESCC are undergoing (Fig. 4 and Table 1) and clinical efficacy has been observed. Combination of chemoradiation with erlotinib has been shown to be effective and tolerable in ESCC patients in phase II studies[112,113]. Among the 21 patients treated, 8 (38%) achieved a complete response, 10 (47.6.%) a partial response and 3 (14.4%) stable disease. No patient showed progressive disease[113]. In a randomized phase III trial for locally advanced ESCC patients, the addition of erlotinib significantly improved overall survival compared with chemoradiotherapy alone (median, 39.4 *vs*. 27.4 months)[114]. Icotinib alone or combined with radiotherapy also showed favorable activity in ESCC patients with EGFR overexpression or amplification. The objective response rate and disease control rate was 16.7% or 46.3%, respectively, in a phase II trial of icotinib monotherapy in ESCC patients with EGFR overexpression[115]. In another randomized phase II trial with 127 ESCC patients enrolled, icotinib plus radiotherapy improved the median overall survival compared with radiotherapy alone (24.0 months *vs*. 16.3 months)[116]. Complete or partial responses were observed in 18 of 20 elderly ESCC patients in a phase II trial of combined radiotherapy and gefitinib[117]. In summary, targeting EGFR is a promising therapeutic option for ESCC patients and *EGFR* amplification may serve as a biomarker indicating favorable response.

3.2.2. *FGFR*

FGF/FGFR participate in multiple cellular processes, whereas deregulation of FGF/FGFR signaling pathway is correlated with developmental disorders and cancers[118]. FGFR inhibitors are developed in recent years and erdafitinib is the first FGFR inhibitor approved by FDA for the therapy of urothelial carcinoma in 2019. Several other FGFR inhibitors also exhibit favorable activity against multiple solid tumors. The study of FGFR inhibitor on ESCC patients is at the early stage. The selective FGFR4 inhibitor H3B-6527 significantly inhibited the growth of ESCC cells *in vitro* and *in vivo* by blocking PI3K and mitogen-activated protein kinase (MAPK) signaling pathways[119]. FGFR inhibitor AZD4245 could enhance the sensitivity of ESCC cells with high level of active FGFR1 to gefitinib, proposing a potential combination for ESCC treatment[120]. Currently, several clinical trials with FGFR inhibitors for esophageal cancer are underway (Fig. 4 and Table 1).

3.2.3. *PI3Kα*

The mutations and amplification of *PIK3CA* are frequent and associated with poor prognosis in ESCC, indicating PI3Kα is a promising target for ESCC treatment. As the first PI3Kα-specific inhibitor approved by FDA, alpelisib has been proved to be effective in several solid tumors including head and neck squamous cell carcinoma and ESCC[121]. Our previous study has reported that CYH33, a novel PI3Kα-selective inhibitor, displayed significant activity against the proliferation of ESCC cells and the growth of xenografts derived from ESCC cells[122]. CYH33 also sensitized ESCC cells to radiation by abrogating survival signals in tumor cells and tumor microenvironment[123]. The combination of PI3Kα inhibitors with other targeted drugs also demonstrated potent anti-ESCC activity. Combination of alpelisib with a JNK inhibitor, SP600125, displayed a synergistic activity against ESCC *in vitro* and *in vivo*, proposing a new therapeutic strategy for ESCC patients[124]. Concurrent inhibition of PI3Kα and RAS/MEK synergistically inhibited the growth of xenografts harboring $HRAS^{G12S}$ mutation, which was worthwhile to be considered in the treatment of ESCC patients harboring mutation or amplification in *KRAS* or *HRAS*[125]. Alpelisib and CYH33 are currently in clinical trials for the treatment of solid tumors including advanced ESCC (Fig. 4 and Table 1).

3.2.4. *WNT signaling pathway*

The WNT signal transduction cascade is a main regulator of development and a key driver of most types of tissue stem cells[126]. The alterations in WNT signaling pathway possess a fundamental role in cancer development and drug resistance[127]. WNT signaling pathway was reported to be hyperactivated in ESCC[24,57]. The biomarker signature score based on a panel of WNT-related markers (cytoplasmic β-catenin, nuclear c-MYC, nuclear dishevelled segment polarity protein 1 and membrane α-catenin) was associated with ESCC recurrence/death[128]. WNT signaling was also found to be involved in radioresistance by promoting DNA damage repair mediated by a chromatin-associated protein transactivating high-mobility group box 1[129]. These studies indicated that WNT signaling might be a potential therapeutic target for ESCC. LGK974 is a porcupine inhibitor, which inhibits the secretion of WNT and its signaling by blocking the palmitoylation of WNT[130]. LGK974 is currently in clinical trials for the treatment of solid tumors including ESCC (Fig. 4 and Table 1).

3.3. *Therapeutic targets in EAC*

3.3.1. *HER2*

HER2 amplification and overexpression have been found in a number of human cancers, such as breast cancer, gastric cancer and EAC. Currently, HER2-targeted therapy has been applied to the treatment of a few types of cancer overexpressing HER2. The anti-HER2 monoclonal antibody trastuzumab exhibited promising

therapeutic effect in HER2-positive EAC patients. In a phase III randomized trial for HER2-positive gastric or gastric-esophageal junction adenocarcinoma patients, the median overall survival was 13.8 months in trastuzumab plus chemotherapy group compared with 11.1 months in chemotherapy alone group[131]. Considering the favorable clinical efficacy, trastuzumab has been approved by FDA for the treatment of HER2-positive EAC patients in combination of chemotherapy (Fig. 4 and Table 2). Other HER2-targeted drugs are being tested in clinical trials (Fig. 4 and Table 1) and favorable activity against HER2-positive EAC has been observed. Lapatinib, a small-molecule inhibitor of HER-2 and EGFR, has been approved to treat HER2-positive breast cancer. Combined lapatinib and chemotherapy was effective in HER2-positive EAC patients with manageable toxicity[132]. In a phase II study of trastuzumab and pertuzumab with chemoradiotherapy in EAC, complete response rate and overall survival was 34% and 71%, respectively. No unexpected adverse events were observed and patients with HER2 overexpression showed better response, indicating that such combination was promising in HER2-positive patients[133]. Based on the positive results of clinical trials, HER2-targetd drugs displayed great promising in the therapy of HER2-positive EAC.

3.3.2. *VEGFR*

VEGF plays an essential role in the regulation of angiogenesis and lymphangiogenesis, which is vital for tumor angiogenesis and metastasis. Targeting VEGFR has been proven effective in diverse solid tumors including EAC. Ramucirumab is a monoclonal antibody targeting VEGFR and has displayed efficacy in EAC patients. In a phase III trial of ramucirumab monotherapy for gastric or gastric−esophageal junction adenocarcinoma patients, the median overall survival was 5.2 months in ramucirumab group and 3.8 months in placebo group[134]. Besides, ramucirumab in combination with paclitaxel significantly improved overall survival compared with placebo plus paclitaxel in gastric or gastric-esophageal junction adenocarcinoma patients (9.6 months *vs.* 7.4 months)[135]. Ramucirumab has been approved by FDA for treatment for EAC patients in view of the promising clinical results (Fig. 4 and Table 2). Bevacizumab is also a monoclonal antibody against VEGFR being tested in clinical trials (Fig. 4 and Table 1). In a phase II study of bevacizumab combined with chemotherapy in metastatic gastric or gastro−esophageal junction adenocarcinoma patients, the overall response rate was 65% and time to disease progression improved over historical controls by 75%[136]. Small molecule VEGFR inhibitors apatinib also showed favorable antitumor activity in EAC patients and has been approved in China as the third-line treatment for gastro−esophageal junction adenocarcinoma[137]. The clinical trial of apatinib combined with chemotherapy in esophageal cancer patients is now undergoing (Fig. 4 and Table 1).

4. Conclusions and perspectives

Esophageal cancer is one of the most lethal malignancies worldwide, whereas the options of molecularly targeted therapy are very limited. At present, there are only three targeted drugs have been approved for clinical applications (Table 2). With the evolution of DNA sequencing technology, the landscape of esophageal cancer genome is being revealed in recent years. We analyzed the genomic data and found that these recurrently altered genes are enriched in regulators of cell cycle (*TP53*, *CDKN2A*, *CDKN2B*, *CCND1* and *MYC*), members in the RTK-MAPK/PI3K pathway (*FGFs*, *EGFR*, *HER2*, *VEGFA*, *PIK3CA*, and *KRAS*), and epigenetic modulators (*KMT2C*, *KMT2D* and *ARID1A*) (Fig. 3). These alterations could disrupt normal epithelia homeostasis and contribute to tumorigenesis and promotion of esophageal cancer.

Though current sequencing technology have provided bulk genomic data to guide the treatment of esophageal cancer, how to select the genomic data due to the intratumoral heterogeneity is still a considerable challenge in clinical practice. Emerging strategies such as multiregional sequencing, single-cell sequencing and analysis of liquid biopsy samples have been introduced. These strategies may help better understand the tumor heterogeneity and provide more accurate data for precise medicine. However, bulk genomic data are usually utilized in the clinical practice due to the cost and efficiency. With further development of the sequencing technology, more precise genomic data will be available for the molecularly targeted therapy in esophageal cancer.

ESCC and EAC are two subtypes of esophageal cancer with diverse epidemiology and pathogenesis as well as different molecular profiles, which provide distinctive targets for the treatment. Further preclinical studies and clinical trials are in progress and some encouraging clinical efficacy has been observed (Fig. 4). Moreover, the combination of targeted drugs and chemotherapy/radiotherapy displayed great potential for this deadly disease. Combination regimens with improved efficacy and reduced toxicity are being tested in clinical trials (Table 1). With the increasing knowledge of molecular characteristics in ESCC and EAC, more molecularly targeted therapy will be developed to benefit the esophageal cancer patients in the near future.

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Author contributions

Linghua Meng conceived the project and revised the manuscript. Xu Zhang and Yuxiang Wang summarized the literature and composed the manuscript. All authors proofread the manuscript and approved to submit the final manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2021.09.028.

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