## Review Article
# Heterogeneous genomic aberrations in esophageal squamous cell carcinoma: a review

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Abstract: Esophageal cancer (EC) causes hundreds of thousands of deaths a year worldwide, especially the major subtype esophageal squamous cell carcinoma (ESCC). With the advent of next-generation sequencing and the availability of commercial microarrays, abnormities in genetic levels have been revealed in various independent researches. High frequencies of structure variations (SVs), single nucleotide variations (SNVs) and copy-number alterations (CNAs) in ESCCs are uncovered, and ESCC shows high levels of inter- and intratumor heterogeneity, implying diverse evolutionary trajectories. This review tries to explain the pathogenesis of ESCC on the scope of most often mutated genes based on prior studies, hopes to offer some hints for diagnosis and therapy in clinic.

Keywords: ESCC, genomic alterations, heterogeneity, evolution, pathogenesis

## Introduction

The esophagus is a hollow, muscular tube consisting of several layers. Esophageal cancer (EC) usually starts on the inner side of the mucosa and spreads outward through the other layers as it grows. According to the Global Cancer Statics 2018, about 572,000 people worldwide are diagnosed with EC a year, which ranked eighth among all new cancer cases, and approximately 509,000 patients die from it, ranking as sixth for cancer mortality [1].

The two most common forms of EC are esophageal squamous cell carcinoma (ESCC), most often found in the upper and middle parts of the esophagus, and esophageal adenocarcinoma (EAC), that usually forms in the lower part of the esophagus, near the stomach. ESCC is the most common subtype, accounting for 90% of all esophageal cancers worldwide [2]. It is different from EAC in terms of its pathogenesis, risk factors, genomic landscape and cellular transcriptome profile [3-5]. There is great variability in the incidence of EC with regard to geography, ethnicity, age, and gender (**Figure 1**)

[6]. For example, In Europe and the United States the predominant histological subtype is EAC, while EAC remains rare in Asia. In China, EC is the third most frequently diagnosed cancer and the fourth leading cause of death from cancer [7]. The incidence in rural areas is more than that in urban areas [7, 8], especially in regions such as Henan, Hebei, and Shanxi [9].

EC is associated with a poor prognosis, despite advances in diagnosis and treatment, the overall 5-year survival rate is less than 20% worldwide [10]. As shown in **Table 1**, metastasis to lymph nodes and other organs markedly lowers the survival rate.

### Genomic alterations in ESCC

With the development of sequencing technology, whole-genome sequencing (WGS), whole-exome sequencing (WES), and transcriptome sequencing (RNA-seq) have been performed in many studies. In addition, various commercial microarrays have been developed and applied to ESCC. As shown in **Tables 2** and **3**, abnormalities in the molecular levels have been uncovered [11-18], providing comprehensive

**Esophageal Cancer Rates By Age**
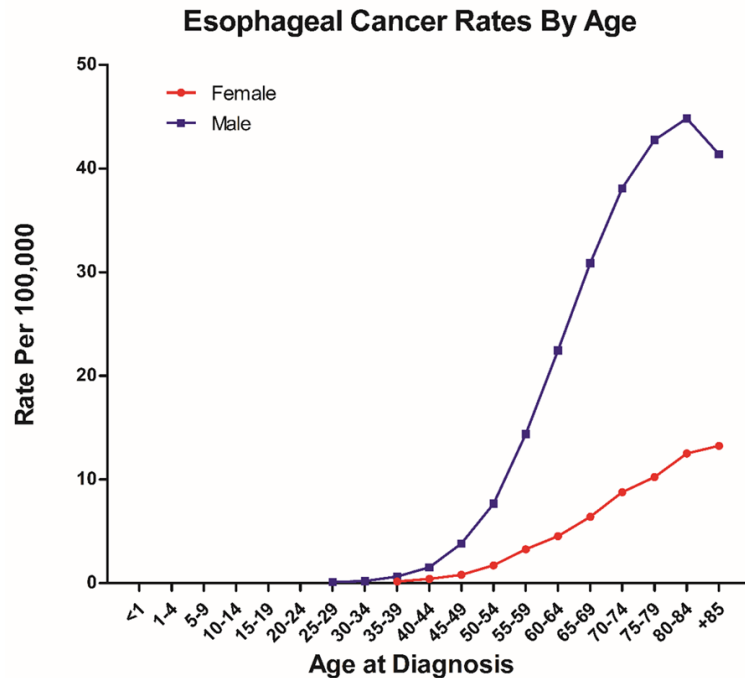


**Figure 1.** Based on SEER (Surveillance, Epidemiology, and End Results) website, esophagus cancer incidence rates by age at diagnosis in USA from 2012 to 2016.

**Table 1.** According to the SEER database, the 5-year survival rates of 21583 cases of esophageal cancer between 2009 and 2015 in the United States, based on how far the cancer has spread

| Stage | 5-Year Relative Survival Rate of EC | 5-Year Relative Survival Rate of ESCC |
|---|---|---|
| Localized | 46.7% | 31.2% |
| Regional | 25.1% | 23.8% |
| Distant | 4.8% | 6.3% |
| All stages combined | 19.9% | 17.6% |

insights into the mutational signature of ESCCs and facilitating identification of biomarkers for early diagnosis and potential therapeutic targets.

Despite the fact that the presence and frequencies of gene alterations in ESCC vary greatly within studies because of geographic or ethnic differences of patients, stages and grades of tumors, the number of tumor samples, inter and intra-tumor heterogeneity, and depth of sequencing [19], some of the findings are consistent.

Numbers of structure variations (SVs) such as large-scale chromosome changes, gene copy number alterations (CNAs), small insertions and deletions (indels), and single-nucleotide variants (SNVs) have been revealed. Among all SNVs, there are high frequencies of C>G, C>T mutations at TpCpX, which are mostly attributed to the hyperactivity of APOBEC family of DNA deaminases [20], and C>T mutation at XpCpG, which may be the result of higher methylation levels and DNA repair inefficiency in tumors [21].

As a result, many genes are affected in ESCCs. The notable recurrent alterations are mutations of *TP53*, *CDKN-2A, NFE2L2, NOTCH1, PIK3-CA, RB1, FAT1, MLL2, FBXW7* and *ZNF750*, amplification of *CCND1* (11q13.3)*, MYC* (8q-24.21)*,* and *SOX2* (3q26.33), and deletion of *CDKN2A* (9p21.3). These aberrations are also involved in the progression of head and neck squamous cell carcinoma (HNSCC) [22] and lung squamous cell carcinoma (LSCC) [23]. In addition, as an amplicon at 13q13.3, *MIR548K, ANO1, FADD, CTTN* and *SHANK2* are also amplified frequently but talked less in previous studies, they all play roles in the progression of ESCC. All these genes orchestrate many other genes to regulate cellular activities and maintain homeostasis. Thus, the disruption of the balance contributes to many cancers through various mechanisms like the discussion below.

*Cell-cycle regulation*

*TP53* is a tumor suppressor gene, its protein product, p53, plays very important roles in cell cycle regulation [24], DNA repair [25], genomic stability maintenance [26], senescence [27], and apoptosis [28]. Various kinds of *TP53* mutations have been found in numbers of human cancers, and it is the most frequently mutated gene in ESCC, with frequent protein loss mutations such as frameshift indels, nonsense mutations, and mutations at splice site. The majority of missense mutations are distrib-

**Table 2.** Recent studies of ESCC using high-throughput sequencing

| Study | ESCC cases | District | Main Methods | Chromosome change | |
|---|---|---|---|---|---|
| | | | | Amplification | Deletion |
| [11] | 158 | Chaoshan, Guangdong, China | WGS (17), WES (71), array-CGH (123) | Large scale amplification of 3q, 5p, 8q, 12p, 20p and 20q; 43 focal CNAs, like 8q24.3, 11q13.3-13.4, 14q32.33 | Large scale deletion of 3p, 4q, 9p, 13q, 18q, 19p and 21q; 15 focal CNAs, such as 9p21.3, 3q26.1 |
| [12] | 220, (103)[1] | North-central China | WES (139), RNA-seq (4), SNP-array (22), array-CGH (59) | 8p11.23, 8q24.21, 7p11.2, 11q13.2-13.3, 12p12.1, 12q15, 3q26, 3q26.32-33, 14q13.3 | Homozygous deletion of 2q22.1-22.2, 9p21.3, 5q12.1, 9p24.1, 3p14.2 |
| [13] | 113 | China | WES (113) | Recurrent CNAs like 11q13.3, 11q13.2, 11q13.4, 11q13.1, 7p11.2, 7q31.1 | Recurrent CNAs like 9p21, Yp11.3, Yq11.222 |
| [14] | 104, (88)[2] | North-central China | WGS (14), WES (90), FISH, qPCR | Recurrent CNAs like 3q26.1-q29, 3q29, 3q25.1, 11q13.3-q13.4, 11q13.3, 8p12-p11.23, 7p12.1-p11.2 | Recurrent CNAs like 9p21.3, 19p11, 5p15.33, 21p11.2, 3p11.1, 4p11, Xq11.1 |
| [15] | 388, (70)[3] | Guangdong, China | WGS (10), WES (60), IHC (120) | Recurrent CNAs like 3q26.33, 11q13, 20q13.12 | Recurrent CNAs like 2q22.1-22.2, 9p21.3, 13q13.3-q14.11 |
| [16] | 144 | Japan | WES SNP-array | 3q, 8q, 5p, 7p, 20q; recurrent focal CNVs, like 11q13.3, 5p15.33, 7p11.2, 11q22.1, 3q26.33 | 3p, 4p, 9p, 5q; recurrent focal CNVs, like 9p21.3, 2q22.1-22.2, 4q22, 3p14.2 |
| [17] | 90 | 10 counties, such as USA, Korea, Brazil, Germany | WES, SNP-array | CNAs, like 3q26.33, 3q28, 5p15.33, 7p11.2, 8p11.23, 11q13.3, 12p12.1, 12q14.3, 14q13.2 | CNAs, like 13q14.2, 3p25.2, 9p21.3, 2q22.1, 8p23.2, Xp11.3, 10p11.21, 3p14.3, 4p15.2, 4q22.1, 3p14.2 |
| [18] | 94 (704, 94)[4] | China | WGS | 3q, 5p, 7p, 8q, 12p, 16p, 20p and 20q; 23 recurrent focal CNVs, like 11q13.3, 3q26.32, 5p15.33, 8q24.21, 7q22.1, 7p11.2, 19p13.3, 14q13.3 | 3p, 4p, 4q, 5q, 9p, 13q, 18q and 21p; 34 focal CNVs, like 9p21.3, 22q11.21, 10p11.22, 5q13.2, 6p24.3, 10q21.1, 12q23.1, 13q31.3 |

WGS: Whole-genome sequencing; WES: Whole-exome sequencing; TS: Targeted sequencing; CGH: Comparative genomic hybridization; RNA-seq: RNA sequence; SNP: Single nucleotide polymorphism; IHC: immunohistochemistry; 1: 44 SNP-array data from GEO: GSE17958, 30 from GSE20347, 29 from GSE19399. 2: 88 Chaoshan sequencing data from [10]. 3: 53, 17 microarray data from GSE23400, GSE20347, respectively. 4: 704 sequencing data from [27-32], and 94 from TCGA

**Table 3.** Recurrent affected genes in recent studies of ESCC

| Pathway | Gene | Location | Origin function on tumor | [11] | [12] | [13] | [14] | [15] | [16] | [17] | [18] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell cycle | TP53 | 17p13.1 | - | S | S | S | S | S | S | S | S |
| | CDKN2A/B | 9p21.3 | - | S, L | S, L | S, L | S, L | S | S, L | S | S, L |
| | RB1 | 13q14.2 | - | S, L | S | S | S | | | L | F |
| | CCND1 | 11q13.3 | + | G | G | G | | G | G | G | G |
| | CCND2 | 12p13.32 | + | G | | | | | | | |
| | CDK4 | 12q14.1 | + | G | | | | | | | |
| | CDK6 | 7q21.2 | + | G | | | | | | G | |
| | E2F1 | 20q11.22 | - | G | | | | | | | |
| | MDM2 | 12q15 | + | G | G | | | | | G | G |
| | TP63 | 3q28 | + | | G | | | | | G | |
| | TP73 | 1p36.32 | + | | | | | G | | | G |
| | MYBL2 | 20q13.12 | + | | | | | G | | | |
| Apoptosis | BCL2L1 | 20q11.21 | | G | | | | | G | | |
| | FADD | 11q13.3 | | G | | G | G | G | G | G | G |
| | TRAF3 | 14q32.33 | | | | | | | | | G |
| RTK-MAPK-PI3K signaling | FGFs | 11q13 | + | G | G | G | G | G | | G | |
| | TGFBR2 | 3p24.1 | - | | | | | | S | S | F |
| | EGFR | 7p11.2 | + | G | G | G | | | G | G | G |
| | FGFR1 | 8p11.23 | + | G | G | | | | G | G | |
| | ERBB2 | 17q12 | + | | O | | | | M | | |
| | KRAS | 12p12.1 | + | G | G | | F | | | G | |
| | MYC | 8q24.21 | + | G | G | G | | G | | G | G |
| | PIK3CA | 3q26.32 | + | S, G | S, G | F | S | | S | G | F, G |
| | AKT1 | 14q32.33 | + | G | | | | | | | G |
| | PTEN | 10q23.31 | - | | S, L | | | | | M | F |
| JAK-STAT signaling | IL7R | 5p13.2 | + | G | G | | | | | | |
| | JAK1 | 1p31.3 | | L | M | | | | | | |
| | STATs | | | G | O | | | | | | |
| | SOX2 | 3q26.33 | + | G | G | G | G | G | G | G | G |
| Notch signaling | NOTCH1 | 9q34.3 | - | S, G | S | S | S | S | | S | S |
| | NOTCH3 | 19p13.12 | - | | | | | | S | | F |
| | RBPJ | 4p15.2 | - | | | | | | S | | F |
| Hedgehog signaling | PTCH1 | 9p22.32 | | | | | F | F | | | |
| | SUFU | 10q24.32 | - | | | | M | | | | |
| Hippo/Wnt signaling | FAT1 | 4q35.2 | - | | S, D | F | S | | S | | F |
| | FAT2 | 5q33.1 | - | | S | M | | | | | |
| | FAT3 | 11q14.3 | - | | | F | | | | | |
| | YAP1 | 11q22.1 | + | G | | G | | | G | G | G |
| | AJUBA | 14q11.2 | - | | | S | S | | S | | S |
| | DVL3 | 3q27.1 | + | G | | | | | G | | G |
| | LRP5/6 | 12p13.2 | + | G | | | | | | | G |
| Chromatin regulator | MLL2 (KMT2D) | 12q13.12 | - | F | S | S | | | S | S | F |
| | MLL3 (KMT2C) | 7q36.1 | - | F | F | | | | | F | |
| | ASH1L | 1q22 | | F | | | | | | | |
| | SETD1B | 12q24.31 | | F | | | | | | | |
| | CREBBP | 16p13.3 | - | F | | F | S | | S | | F |
| | EP300 | 22q13.2 | - | F | S | F | | | S | | |
| | ZNF750 | 17q25.3 | - | | S | | S | S | | S, L | F |
| | ARID2 | 12q12 | - | | F | | | | | | |
| | PBRM1 | 3p21.1 | - | | F | | | | | | |
| | KDM6A | Xp11.3 | - | | S | F | | | | F, L | |
| | BAP1 | 3p21.1 | - | | | | S | | | | |
| Oxidative response | NFE2L2 | 2q31.2 | + | S | S | S | S | S | S | S | S |
| | KEAP1 | 19p13.2 | | | M | | | | | | |
| | CUL3 | 2q36.2 | - | | M | | | | | | F |

| Category | Gene | Cytoband | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Other | PTPRD | 9p24.1-p23 | - | | L | | | | | | |
| | PDE4D | 5q11.2-q12.1 | - | | L | | | | | | |
| | LRP1B | 2q22.1-22.2 | | L | L | | | L | | | S |
| | FAM135B | 8q24.23 | + | S | | | | | | | |
| | ADAM29 | 4q4.1 | | S | | | | | | | |
| | FBXW7 | 4q31.3 | - | | F, D | S | S | | S | | S, L |
| | CBX4/8 | 17q25.3 | | G | | | G | | | | |
| | TERT | 5p15.33 | + | G | | | | | G | G | G |
| | NKX2-1 | 14q13.3 | + | G | G | | | | | G | G |
| | FHIT | 3p14.2 | - | | L | | | | L | L | L |
| | TET2 | 4q24 | - | | | | | | S | | |
| | TTN | 2q31.2 | | G | | | | S | | | |
| | MIR548K | 11q13.3 | + | G | G | G | G | | G | G | G |
| | SHANK2 | 11q13.3-q13.4 | | G | G | G | G | G | G | | G |
| | CTTN | 11q13.3 | + | G | G | | G | G | G | G | G |
| | ANO1 | 11q13.3 | + | G | | G | G | G | G | G | G |

+: tumor promotion. -: tumor suppression. S: SMG, significantly mutated gene; F: frequently changed gene; G: gain of gene; L: loss of gene; O: overexpression; D: down-regulation.

uted in the DNA binding domain (amino acids 102-292) [29]. The classical G1/S phase checkpoint in the cell cycle is mediated through p16 (*CDKN2A*)-cyclin D (CCND1,2)-Rb and MDM2-p53-p21 (*CDKN1A*) pathways [30]. Functional loss of *TP53*, *CDKN2A* or *RB1*, and amplification of *CCND1* lead to dysregulation of the cell cycle. In addition, other genes contributing to G1/S transition are also amplified in ESCCs, such as *CDK4*, *CDK6*, *E2F1*, and *MDM2*, although at much lower frequencies. Apart from the loss of the DNA-binding capability, such as mutations at residues R175, R248 or R273 [29], accumulating evidence has shown gain-of-function missense mutations of *TP53*, which drive tumor progression through different mechanisms [31]. Among them, p.R175H and p.R273H mutants not only lose tumor suppressor activity but also promote transformation and metastasis through the gained function of promoting integrin recycling [32], and both mutations have been found in ESCC by WGS and WES.

*RTK-MAPK-PI3K signaling pathway*

The binding of growth factors to their receptor tyrosine kinases (RTKs) could stimulates both PI3K-AKT and MAPK signaling pathways [33]. Activated Akt controls important cellular processes by phosphorylating substrates involved in apoptosis, protein synthesis, metabolism, migration, and cell cycle to promote cell survival and progression [34]. Activated ERK phosphorylates other factors such as c-Myc to enhance cell proliferation and differentiation [35]. Also, the aberrant activation of the RTK-MAPK-PI3K pathway is quite often detected in cancers. For Example, *PIK3CA* has been reported to be mutated quite frequently in various cancers, and the missense substitutions show a non-random distribution with hotspots at the helical domain (exon 9, commonly E542 and E545) and kinase domain (exon 20, commonly H1047), implying increased kinase activity of these mutants [36]. The three explicit oncogenic mutants p.E545K, p.E542K and p.H1047R [37] all have high frequencies in the current ESCC sequencing analyses. Apart from gain-of-function mutations of *PIK3A,* amplification or overexpression of *MYC*, *FGFs*, *EGFR*, *FGFR1* and *KRAS*, and loss-of-function mutations of *PTEN* contribute to the survival and progression of ESCC.

*NFE2L2*

Reactive oxygen species (ROS) are highly cytotoxic, one of the cell's defending strategies is to detoxicate ROS by encoding more than 20 phase II detoxifying enzymes [38]. The expression of the enzymes is regulated by the antioxidant response element (ARE)-binding transcriptional complex, and NF-E2-related factor 2 (Nrf2), the protein product of *NFE2L2*, is an essential component of the complex [39]. Besides the cytoprotective role, Nrf2 participates in many cellular activities like cell differentiation and metabolism, it can suppress carcinogenesis in early stages while promote tumor growth and metastasis in later stages [40]. A research has confirmed that oncogenic Nrf2

mutations is a feature of SCC and promote the development of various kinds of SCCs [41], which maintains high protein levels by inhibiting Keap1-Cul3-Roc1-dependent proteasomal degradation of Nrf2, such as mutations at DLG motif ([23]LxxQDxDLG[31]) or ETGE motif ([77]DxETGE[82]) [42]. In a recent EC cancer research, Nrf2 was confirmed to strongly promote glutathione metabolism, offering essential fuels for cancer progression [43]. Overall, gain-of-function mutation or higher expression of *NFE2L2* is associated with tumor recurrence, poor prognosis, and resistance against chemoradiation therapy in ESCC [44].

### FBXW7

*FBXW7* is one of the most frequently mutated tumor suppressor genes in human cancers, which encodes the ubiquitin ligase FBW7 that targets many oncoproteins for ubiquitin-mediated protein degradation. The well-studied substrates involved in tumor progression are cyclin E, c-Myc, and c-Jun [45-48], thus, the aberrant function of FBW7 lead to the accumulation of numbers of oncoproteins to promote tumorigenesis in synergetic ways.

### NOTCH1

Notch signaling is a highly evolutionarily conserved pathway implicated in cell fate determination, proliferation, and apoptosis [49]. It can play both tumor promotive and suppressive roles depending on the cancer cell type [50]. Quite a large fraction of *NOTCH1* mutations in head and neck squamous cell carcinoma (HNSCC) are predicted to truncate the gene product or occur in the TAD domain required for transactivation of target genes, suggesting that Notch1 functions as a tumor suppressor in this type of tumor [51]. Similarly, the mutations of *NOTCH1* in ESCCs are found to be stop-gain SNVs, frameshift indels, at splice sites, or harmful missense SNVs, implying a tumor suppressor role the functional protein plays in esophageal squamous cell. Activated Notch1 was shown to engage in cell cycle arrest by up-regulation of p21 and p27 in small cell lung cancer cells [52]. In human ESCC cell line EC9706, ectopic expression of Notch1 showed up-regulation of GSK3β and down-regulation of β-catenin, implying that Notch signaling inhibited proliferation and induces apoptosis through Wnt signaling pathway [53], though the mechanism remains to be elucidated.

### SOX2

SOX2 is an indispensable factor for the induction of pluripotent stem cells in vitro [54]. It plays different and even opposite roles in various cancer types [55, 56], in SCC, it has been proven to be essential for tumor initiation and cancer stem cell [57], and be an amplified lineage survival oncogene in lung squamous cell carcinoma (LSCC) and ESCC, but alone, it was insufficient to transform epithelial cell lines in vitro, implying co-operation with other signals to promote proliferation and metastasis [58]. As a transcription factor, SOX2 promotes tumorigenesis and tumor progression through diverse mechanisms. One was proved to be linked to the activation of the PI3K/AKT/mTOR1 signaling pathway [59], one was owed to epithelial-mesenchymal transition (EMT) through activation of STAT3/HIF-α signaling [60], and another was to promote the expression of SOX2 overlapping transcript (SOX2OT), an important lncRNA that in turn promoted the growth of ESCC [61]. Notably, ESCC patients with high expression levels of *SOX2* had favorable prognosis, suggesting that it could serve as a prognostic factor and therapy target in clinic [62, 63].

### FAT1

*FAT1* belongs to the cadherin superfamily, whose ortholog in Drosophila encodes a tumor suppressor essential to control cell proliferation during development [64]. FAT1 plays an essential role in cellular polarization, directs cell migration, and modulates cell-cell contact [65]. It was found to suppress multiple cancer cell growth in vitro and in vivo through quenching Wnt signaling though binding β-catenin to antagonize its nuclear localization [66]. In studies of HNSCC, functional loss of FAT1 resulted in YAP1 activation [67], promoted invasion of HNSCC cell lines, and was associated with nodal involvement, lymphovascular permeation and tumor recurrence [68]. In ESCC studies, *FAT1* mutation disrupted the MAPK/ERK pathway to contribute to EMT [69], and affected cytoskeletal proteins to alter cellular mechanical properties, leading to deregulation of cell migration and invasion of ESCC cells [70].

### AJUBA

*AJUBA* encodes a LIM domain-containing protein that acts as a scaffold protein [71], and

shuttles between cytoplasm and nucleus to engage in many cellular activities. Generally, it is considered as a tumor suppressor gene. Ajuba is highly expressed in epithelial cells and interacts directly with E-cadherin and F-actin to mediate cell-cell junction formation [72]. In addition, it does not interact with F-actin but acts with Grb2 to augment MAPK signaling in fibroblasts that have much lower Ajuba expression level [73]. Within the nucleus, Ajuba can work as a co-repressor with transcriptional repressor protein Gfi1 to auto-regulate *Gfi1* expression in 293T and myeloid cell lines [74]. Ajuba specifically associate with LATS/Wts and WW45/Sav in insect and mammalian cells to antagonize the phosphorylation of YAP1 [75], a downstream nuclear effector of the highly conserved Hippo signaling pathway. Moreover, Ajuba acts as a negative mediator of Wnt signaling by promoting GSK3β-mediated phosphorylation of β-catenin, that subsequently undergoes proteasome-dependent degradation [76]. Overall, loss-of-function mutations of *AJUBA* promote tumorigenesis.

*Genes at 13q13.3*

*MIR548K* encodes a microRNA miR-548k that regulates the expression of many proteins, the role it plays in ESCC has been revealed recently. It has been reported to reduce lncRNA-LET expression and increase NF90 expression in a feedback loop, which results in decreased p53 expression and increased HIF-1α and VEGF to enhance ESCC proliferation [77, 78]. Besides, MiR-548k down-regulates ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin motifs 1), resulting in increased secretion of VEGFC and tyrosine phosphorylation (p-Tyr) of VEGFR3 to promote lymphangiogenesis and lymph node metastasis. Additionally, miR-548k represses KLF10, an EGFR transcriptional repressor, to promote metastasis of ESCC cells [79]. Thus, serum abundance of miR-548k and VEGFC may be promising biomarkers for diagnosis of ESCC [79].

*ANO1* encodes a $Ca^{2+}$-activated $Cl^-$ channel, that is widely expressed and plays diverse functions in various tissues [80]. It is amplified or highly expressed in a variety of carcinomas, indicating an oncogenic role [81]. Overexpression of ANO1 enhances EGFR signaling and CAMKII (calmodulin-dependent protein kinase

II) signaling in breast cancer [82], and inhibits apoptosis via downregulation of Bim in HNSCC [83]. In esophageal epithelial cell, the expression level of ANO1 was confirmed to highly corelate with the level of p63 and promote cell cycle, being rational to serve as a therapeutic target [84].

*CTTN* encodes an actin-associated scaffolding protein, that binds and activates the Arp (actin-related protein) 2/3 complex to regulate the formation of dynamic cortical actin-associated structures [85]. Ectopic expression of *CTTN* increases motility and invasion of tumor cells and correlates with metastasis of HNSCC [86]. Moreover, amplification and overexpression of *CTTN* correlates with lymph node metastasis by activating the PI3K/Akt pathway to inhibit anoikis in ESCC [87]. Thus, *CTTN* may serve as a candidate prognostic indicator and therapeutic target in ESCC [88].

*SHANK2* also encodes a scaffolding protein, that organizes the postsynaptic density region in neurons and is related to many neuron disorders like autism [89]. In addition, Shank2 interacts with and activate NHE3 (Na+/H+ exchanger 3), involved in the fine regulation of transepithelial salt and water transport [90]. Furthermore, a truncated isoform, Shank2E, is highly expressed and associates with cortactin and actin at the apical membrane of liver epithelial cells [91]. However, the role of *SHANK2* amplification in the development of tumors remains elusive.

FADD, the protein product of *FADD*, is a well-known key adaptor protein that transmits apoptotic signals mediated by death receptors [92]. In line with other scaffolds, it plays multifunctional roles in cells [93], such as regulating glucose and fat metabolisms [94]. In tumors, FADD was demonstrated to be required for Kras-mediated tumorigenesis in a murine model of lung cancer [95], as well as pancreatic cancer cell proliferation and drug resistance [96]. Amplification of FADD together with other genes was believed to contribute to the progression of HNSCC [97], but the role it plays in ESCC remains obscure.

*Epigenetic regulation*

Epigenetic regulation determines cell fate and dysregulation causes pathologies and diseas-

es [98]. Modifications of histone proteins and changes of DNA methylations are the two major ways to regulate gene expression.

MLL2 (KMT2D) and MLL3 (KMT2C) belong to the KMT2 family, which methylate lysine 4 on the histone H3 tail to promote transcription [99]. Downregulation of MLL3 promotes tumorigenicity of ESCC cells, indicating a tumor suppressor role in ESCC [100]. MLL2 orchestrates H3K27 acetyltransferase p300 to promote differentiation [101], therefore, both MLL2 and p300 are tumor suppressors and are frequently mutated in many types of human cancers [102], which is consistent with the majority of their loss-of-function mutations in ESCCs.

ZNF750 encodes an important transcription factor that acts downstream of p63 to induce KLF4 and drive terminal epidermal differentiation [103]; Moreover, ZNF750 interacts directly with many chromatin regulators, such as RCOR1, KDM1A and CTBP1/2, to induce differentiation [104], it is regarded as a crucial SCC-specific tumor suppressors for its differentiation-induction and migration-reduction functions [105]. In oral squamous cell carcinoma, ZNF750 was shown to modulate the vascular microenvironment by regulating expression of many genes to inhibit malignant progression of tumor cells [106]. Overall, ZNF750 is regarded as a tumor suppressor gene in ESCC, and its low expression may be a biomarker for lymph node metastasis and be linked to poor prognosis [107].

Despite infrequent inactivation in many cancers and ESCCs, H3K27me2/3 demethylase KDM6A is thought to be a tumor suppressor [108], and the function greatly relies on its association with MLL3/MLL4 [109].

CREBBP, the ortholog of p300, is also inactivated in a range of cancer types, supporting it functions as a tumor suppressor [110]. The two acetyl transferases as cofactors participate in regulating the expression of thousands of genes, they are involved in many cellular activities like cell cycle regulation and DNA damage repair [111, 112].

In ESCCs, large-scale chromosome deletion always occurs in 3p, and the 3p21 locus harbors three tumor suppressor genes: PBRM1, BAP1 and SETD2; In particular, SETD2 is responsible for H3K36 trimethylation, which directs DNA mismatch repair, and its loss triggers microsatellite instability and can increase genome-wide mutation rates [113]. Therefore, deletion of this region is catastrophic and greatly enhances tumor progression.

## High heterogeneity and distinct evolution

Based on many valuable sequencing studies in recent years, the onset and development of ESCC has become clearer. As early as 40 years ago, esophageal dysplasia was believed to be the precursor lesion of ESCC [114], and the risk of developing ESCC was in parallel with the grade of dysplasia [115]. Until recently, this phenomenon was given a reasonable explanation. That was mutational burden, including gene mutations and CNAs, was already present in the physiologically normal esophageal epithelia (PNE) [116], and increased with dysplasia progression, reaching the top in high-grade dysplasia and ESCC [117, 118]. Besides, high heterogeneity of gene mutations and CNAs existed among dysplasia and tumors from the same patient, showing highly distinct evolutionary trajectories [117, 118]. In addition, the study revealed that inflammation promoted mutation and aggravated both dysplasia and carcinoma [118]. All these works highlighted the two genetic hits of TP53, which should play pivotal roles in tumorigenesis of ESCC. Nevertheless, the studies showed that the mechanisms of positive selection in PNE versus ESCC differed greatly, while it showed more similarities between high-grade dysplasia and ESCC.

Apart from the separate sites of abnormal esophageal tissues harboring high heterogeneity, a high level of intratumor heterogeneity (ITH) was also proved to be present in ESCC like many other tumors, showing divergent evolution in many aspects [119]. As high as 90% of all recurrent CNAs from different regions of a tumor were unraveled to be spatially heterogeneous [120], and a wide range of heterogeneously somatic variants were revealed in all researches about multi-region ESCC [119-121]. In addition, ITH of DNA methylation and T cell immune repertoire were also identified in two of the above studies, contributing to the heterogeneity and evolution of ESCC.

Research about clonal evolution of ESCC from normal mucosa to primary tumor and metastasis was conducted in 10 patients recently [122], the results also showed complexity in clones within each patient, both linear and parallel metastatic models were shown to be present in ESCC.

## Discussion

Esophageal cancer, especially the esophageal squamous cell carcinoma subtype has a worldwide poor prognosis and the high death rate is largely attributed to late diagnosis and a lack of effective therapies. With the convenience of sequencing, molecular mechanisms underlying ESCC have been revealed, although the key step for transformation is yet to be determined. Not only the presence of the above recurrent mutated genes is important, but also the order of the occurrence plays critical roles in the development of ESCC. Every mitosis brings the chance of mutation to the genome, as a result, mutation density increases with age. Among the mutations, quite a number of them are passenger mutations that do not offer growth advantage to the host, or some of them offer limited growth promotion function to maintain the physiologically normal esophageal epithelia. With the accumulation of driver mutations, low-grade dysplasia manifests and it progresses to high-grade dysplasia, ESCC and eventually metastasizes to other organs. For its high frequency of mutations both in ESCC and high-grade dysplasia, and as the various phylogenetic trees show, the two-hits event of *TP53* plays pivotal roles in tumorigenesis. Though diverse evolution patterns of ESCC coexist based on phylogenetic tree models, which is in accordance with studies of other cancer types, commonalities can still be inferred from the highly heterogeneous phylogenetic trees of ESCC. Driver mutations of tumor suppressor genes are more likely to be located at the trunk of phylogenetic trees than those of oncogenes, such as *TP53* and *CDKN2A*. Amplification of 3q (*SOX2*) and 11q (*CCND1*), and the loss of 9p (*CDKN2A*) also tend to be early driver events in carcinogenesis.

Despite of the many known genomic alterations in ESCC through sequencing technology, we are far from the understanding of the dynamic process of tumorigenesis. Along with continuous evolution, both spatial and temporal heterogeneities exist in ESCC, contributing to the complexity and difficulty of researches in the development of ESCC. Besides, the huge number of tumor cells in a single tumor are not present as isolated ones, they communicate and even cooperate with each other and thus behave as a community to support and promote tumorigenesis [123]. Moreover, tumor cells co-evolve with their micro-environments; extracellular matrix, tumor vasculature and immune cells participate in this process and play multiple roles in carcinogenesis [124].

Many mechanisms such as carcinogens, APOBEC functions, and DNA repair deficiency contribute to genomic instability, which in turn greatly promote to the development and high heterogeneity of ESCC. To get better outcomes in clinic, Both early detection and personalized multi-targeted therapies are in need.

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## Disclosure of conflict of interest

None.

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