

REVIEW

Advances in multi-omics for esophageal squamous cell carcinoma: diagnostic, prognostic, and therapeutic perspectives

Dengyun Zhao^{1,2,3,4,†}, Xinyu He^{2,3,†}, Yaping Guo^{2,5,6, }, Huifang Wei^{2,3}, Zigang Dong^{2,3,4,5,6,7,* }, Kangdong Liu^{2,3,4,5,6,7,*}¹Medical Research Center, Chest Hospital of Zhengzhou University, Zhengzhou 450000, China²Department of Pathophysiology, School of Basic Medical Sciences, Zhengzhou University, Zhengzhou 450000, China³China-US (Henan) Hormel Cancer Institute, Zhengzhou 450000, China⁴Tianjian Laboratory of Advanced Biomedical Sciences, Zhengzhou University, Zhengzhou 450000, China⁵The Collaborative Innovation Center of Henan Province for Cancer Chemoprevention, Zhengzhou 450000, China⁶State Key Laboratory of Esophageal Cancer Prevention and Treatment, Zhengzhou University, Zhengzhou 450000, China⁷Provincial Cooperative Innovation Center for Cancer Chemoprevention, Zhengzhou University, Zhengzhou 450000, China

†These authors contributed equally to this work.

*Correspondence: dongzg@zzu.edu.cn (Z. Dong), kdliu@zzu.edu.cn (K. Liu)

Abstract

Esophageal squamous cell carcinoma (ESCC) remains a major health burden, particularly in Asia, with poor patient prognosis despite advancements in radiotherapy, chemotherapy, and immunotherapy. The marked interpatient and intratumor heterogeneity of ESCC underscores the need for molecularly informed diagnostic and therapeutic strategies. Recent high-throughput omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, have substantially advanced our understanding of ESCC biology. Genomic profiling has revealed recurrent alterations such as *TP53* and *NOTCH1* mutations, as well as actionable targets including *PIK3CA*, *FGFR1*, and *SOX2* amplifications, which provide new opportunities for precision therapy. Epigenomic and transcriptomic analyses have identified methylation-based early detection markers (e.g., *PAX9*, *SIM2*) and immune-related transcriptomic subtypes associated with prognosis and immunotherapy responsiveness. Proteomic and metabolomic studies have further uncovered cell cycle and spliceosome pathway activation and altered lactate metabolism, offering additional biomarker and therapeutic insights. In this review, we synthesize these multi-omics advances and highlight how they collectively inform improved diagnostic, prognostic, and therapeutic strategies for ESCC. Despite these developments, the clinical translation of multi-omics findings remains limited due to the lack of standardized analytical pipelines, insufficient multicenter validation, and the high cost and technical complexity of integrating multi-omics data into routine clinical workflows. Future research integrating artificial intelligence with multi-omics data holds promise for enhancing diagnostic accuracy and enabling more precise therapeutic decision-making in ESCC.

Keywords high-throughput omics, ESCC, mechanism, therapeutic target, diagnosis marker, prognosis marker, tumor microenvironment

Introduction

Esophageal cancer is the eighth most commonly diagnosed malignancy worldwide and the sixth leading cause of cancer-related mortality (Pennathur et al., 2013; Siegel et al., 2016). Survival rates for esophageal cancer remain

low, with 5-year survival rates ranging from 10% to 30% in most countries (Enzinger and Mayer, 2003). Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the two major histological subtypes of esophageal cancer based on histological features

(Morgan et al., 2022). The impact of esophageal cancer varies by country and population, with its prevalence mainly related to underlying risk factors and differences in subtype distribution. While EAC is predominant in Western populations and associated with Barrett's esophagus, gastroesophageal reflux, and obesity (Pohl et al., 2013; Quante et al., 2018), ESCC accounts for approximately 90% of cases globally and is the predominant subtype in Asia, strongly linked to tobacco use, alcohol consumption, and nutritional factors (Rustgi and El-Serag, 2014). Among these two subtypes, ESCC remains the most urgent clinical challenge due to its high incidence, aggressive behavior, and pronounced inter- and intratumoral heterogeneity. Although surgical resection remains the primary curative option (Li et al., 2021a), patients with locally advanced ESCC often experience recurrence or distant metastasis (Matsui et al., 2022). In addition, the substantial heterogeneity of ESCC complicates early detection, risk stratification, and treatment selection, highlighting the need for deeper molecular interrogation to support precision oncology.

High-throughput omics technologies including genomics, epigenomics, transcriptomics, proteomics, posttranslational modification profiling, and metabolomics enable comprehensive characterization of cancer at multiple biological levels. Whereas single-omics approaches provide insights into key molecules and events at a specific level, integrated multi-omics strategies have the unique capability to reveal regulatory interactions, delineate tumor evolution, and identify actionable pathways across genomic, epigenetic, transcriptional, and proteomic layers. For ESCC, multi-omics studies have substantially shifted the research paradigm from single-gene investigation toward systems-level dissection of tumorigenesis, tumor microenvironment (TME) remodeling, and therapeutic vulnerabilities.

In this review, we summarize recent progress in single-omics and multi-omics research related to ESCC, highlight key molecular alterations and regulatory programs uncovered by these technologies, and evaluate their potential diagnostic, prognostic, and therapeutic implications. We also provide a comparative assessment of different omics strategies and discuss how integrated multi-omics analyses contribute to mechanistic understanding and precision treatment development. Despite the rapid accumulation of omics datasets, significant challenges remain in translating these findings into routine clinical practice, particularly due to the lack of standardized analytical workflows, limited multicenter validation, and complexities associated with integrating multi-dimensional data for clinical decision-making.

Genomic alterations in ESCC development

Advances in next-generation sequencing (NGS) and reductions in sequencing costs have greatly accelerated genomic profiling in ESCC, enabling detailed characterization of

single-nucleotide variants (SNVs), copy number alterations (CNAs), and chromosomal rearrangements through whole-exome sequencing (WES) and whole-genome sequencing (WGS). These high-throughput approaches have revealed extensive genomic instability and provided a comprehensive mutational landscape that underpins ESCC initiation, progression, metastatic dissemination, and therapeutic resistance (Fig. 1 and Table 1).

Recurrent genomic loci and chromosomal aberrations drive ESCC susceptibility and instability

Genome-wide association studies (GWAS) in high-incidence Chinese populations have identified multiple susceptibility loci linked to ESCC risk. Significant variants include rs7447927 at 5q31.2 (a single-nucleotide polymorphism [SNP] in TMEM173), rs1642764 at 17p13.1 (an intronic SNP in ATP1B2, near TP53), rs35597309 at 6p21.32 (in the HLA class II region) (Wu et al., 2014b), as well as additional risk loci at 5q11, 6p21, 10q23, 12q24, and 21q22 (Wu et al., 2011b). In a subsequent study, nine additional loci were identified, including seven with significant marginal effect on chromosomes 4q23, 16q12.1, 17q21, 22q12, 3q27, 17p13, and 18p11 (Wu et al., 2012). Additionally, variants such as rs1050631 in SLC39A6 have also been associated with susceptibility and instability (Wu et al., 2013). These findings underscore the role of germline predisposition in shaping population-level ESCC risk.

Frequently mutated genes act as oncogenic drivers

TP53 remains the most frequently mutated gene in ESCC (Cancer Genome Atlas Research Network et al., 2017; Testa et al., 2017; Wang et al., 2015). The Cancer Genome Atlas (TCGA) WES analysis further identified recurrent amplification of CCND1, SOX2, and TP63, which activate pathways critical for squamous differentiation such as WNT, Syndecan, and p63 pathways. Across independent cohorts, recurrent mutations have been identified in TP53, NFE2L2, MLL2, ZNF750, NOTCH1, and TGFBR, highlighting the predominance of alterations in squamous lineage regulators and oxidative stress pathways—features that align ESCC genetically with head and neck squamous carcinoma rather than EAC (Cancer Genome Atlas Research Network et al., 2017).

Deep sequencing studies have expanded the mutational landscape of ESCC. Exome sequencing of 113 pairs of tumor and normal tissues confirmed frequent alterations in cell cycle regulators (TP53, CDKN2A, RB1), stress-response genes (NFE2L2), histone modifiers (KMT2D, KMT2C, KDM6A, EP300, and CREBBP), and components of the Hippo (FAT family) and Notch (NOTCH family) pathways (Gao et al., 2014). A subsequent genomic study of 158 ESCC cases confirmed recurrent mutations in major cancer drivers, including TP53, RB1, CDKN2A, PIK3CA, NOTCH1, and NFE2L2, and discovered novel mutations in ADAM29 and FAM135B, along with additional mutations in histone-regulating genes (KMT2D, ASH1L, KMT2C, SETD1B,

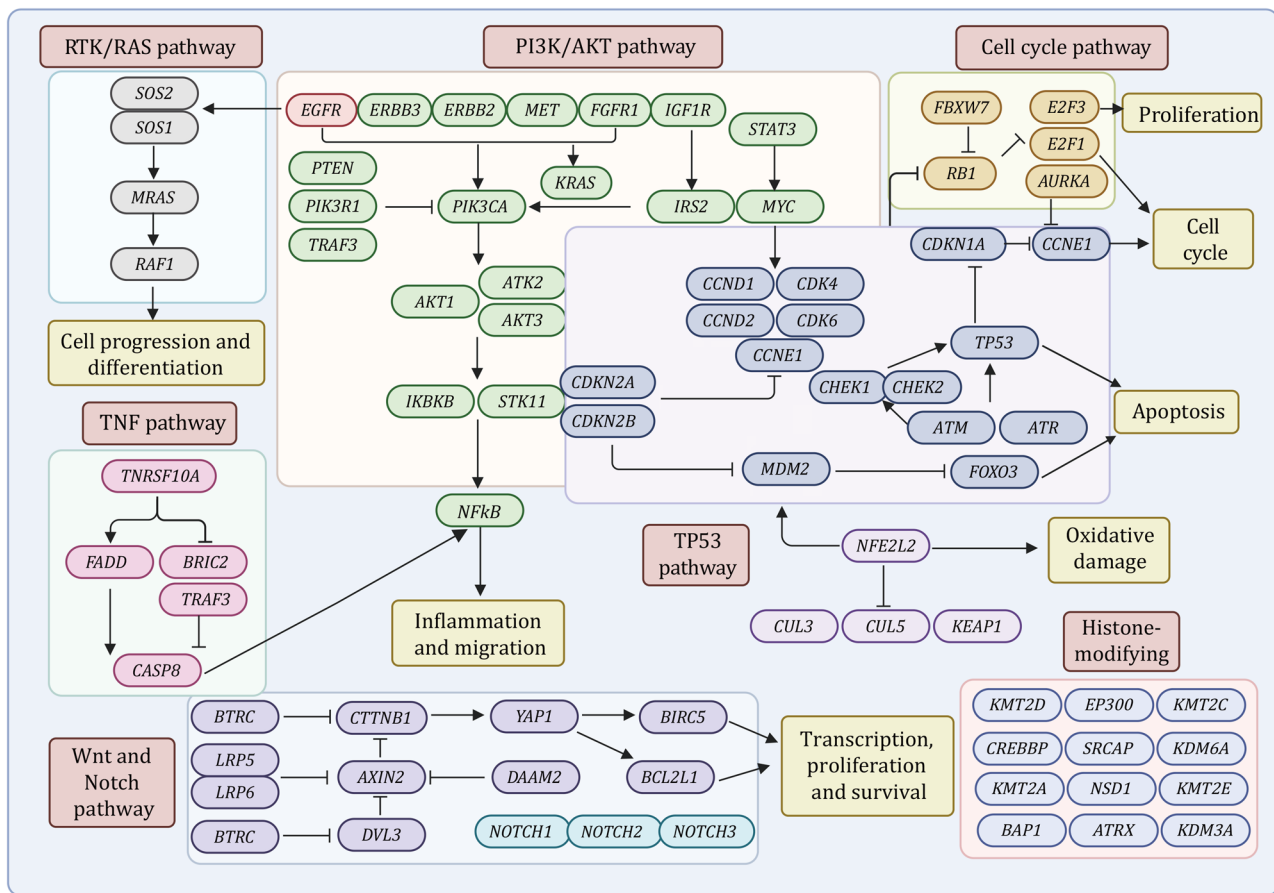


Figure 1. Genomic landscape and core oncogenic signaling pathways in ESCC. Genomic studies have demonstrated that ESCC is driven by recurrent alterations in multiple signaling pathways, including RTK/RAS, PI3K/AKT, cell cycle, TP53, TNF, Wnt, and Notch pathways, together with extensive disruption of histone-modifying. Frequent alterations in genes such as TP53, CDKN2A, CCND1, PIK3CA, EGFR, NOTCH1, NFE2L2, and epigenetic regulators (KMT2D, KMT2C, EP300, CREBBP) collectively promote uncontrolled proliferation, impaired squamous differentiation, cell cycle, inflammatory signaling, and tumor progression.

CREBBP, and EP300) that collectively disrupted WNT, cell cycle, and Notch signaling (Song et al., 2014). Integrated WES and SCNv profiling in larger cohorts further highlighted recurrent mutations in FAT1, FAT2, ZNF750, and KMT2D, and together with aberrations in RTK-MAPK-PI3K, cell cycle regulation, and others (Lin et al., 2014). More recently, WGS of 508 ESCC tumor and paired nontumor tissues identified 22 significantly mutated genes, including both previously recognized drivers (e.g., TP53, FAT1, NOTCH1, KMT2D, FBXW7) and newly implicated genes such as KRT5, CDH10, LILRB3, YEATS2, and CASP8 (Cui et al., 2020). Collectively, these studies reveal a genomic landscape dominated by disruptions in squamous lineage regulators, chromatin remodeling factors, and major oncogenic pathways.

Geographic and ethnic variation shapes ESCC mutational landscapes

Notable regional differences have been observed in ESCC genomic profiles. For example, mutations in AJUBA, ZNF750, FAT1, and FBXW7 were enriched in northern but not southern Chinese populations. Brazilian cohorts showed TP53 and NOTCH1 as dominant mutations (Munari

et al., 2021), whereas Japanese studies reported high frequencies of mutations in TP53, CCND1, CDKN2A, MLL2, NOTCH1/3, FAT1, and PIK3CA (Sawada et al., 2016; Urabe et al., 2019b). Indian ESCC samples frequently harbored TP53, CSMD3, SYNE1, PIK3CA, and NOTCH1 mutations (Mangalaparthy et al., 2020), while Iranian cases displayed mutations linked to DNA repair pathways, cell cycle control, and metabolism related to alcohol, folate, and carcinogens (Akbari et al., 2009). African-American patients exhibited SBS16 and SBS26 as dominant mutational signatures, along with MYC, PIK3CA, SOX2, CCTN, and KRAS alterations (Erkizan et al., 2021). These observations demonstrate that ESCC mutational patterns are shaped by genetic ancestry.

Risk and metastasis-associated mutations

Environmental exposures, particularly alcohol consumption and tobacco use, shape distinct mutational signatures in ESCC. The alcohol-associated E4 signature, characterized by T>C substitutions, correlates with germline variants in alcohol-metabolizing enzymes as well as with alcohol intake (Cui et al., 2020). Consistently, carriers of risk alleles in both ADH1B and ALDH2 who drink alcohol

Table 1. Representation of genetics studies in ESCC.

Cancer type	Platform	Sample sources	Number of samples	Key mutant genes	Key mutant pathways	Function	References
ESCC	WES	Tissue	WES (n = 113)	TP53, CCND1, CDKN2A, NFE2L2, RB1, KMT2D, KMT2C, KDM6A, EP300, CREBBP, FAT1, FAT2, FAT3, FAT4, AJUBA, NOTCH1, NOTCH2, NOTCH3, FBXW7	Cell cycle, apoptosis, histone modifiers, Hippo and Notch pathways	EP300 mutations were associated with poor survival	(Gao et al., 2014)
ESCC	WGS, WES	Tissue, blood	WGS (n = 17), WES (n = 71)	TP53, RB1, CDKN2A, PIK3CA, NOTCH1, NFE2L2, ADAM29, FAM135B, MIR548K, MLL2 (KMT2D), ASH1L, MLL3 (KMT2C), SETD1B, CREBBP, EP300	Wnt, cell cycle, Notch, RTK-Ras and AKT pathways	FAM135B serve as a prognostic marker or therapeutic target	(Song et al., 2014)
ESCC	WES	Tissue	WES (n = 20)	TP53, PIK3CA, NOTCH1, FAT1, FAT2, ZNF750, KMT2D	RTK-MAPK-PI3K, cell cycle and epigenetic regulation pathways	XPO1 and FGFR1 serve as therapeutic targets, ZNF750, FAT1 and FAT2 serve as tumor suppressors	(Lin et al., 2014)
ESCC	WGS	Tissue	WGS (n = 508)	TP53, FAT1, NOTCH1, KMT2D, CDKN2A, FBXW7, ZNF750, FAT2, PIK3CA, EP300, NFE2L2, AJUBA, RB1, KMT2C, CREBBP, KMD6A, TGFB2, KRT5, CDH10, LILRB3, YEATS2, CASP8	RTK-RAS, MYC, cell cycle, NRF2, NOTCH and WNT pathways	NFE2L2 serve as tumor suppressor and prognostic marker, SLC35E2 serve as prognostic marker	(Cui et al., 2020)
ESCC	NGS	Tissue	NGS (n = 46)	TP53, NOTCH1, NFE2L2, RB1, PTEN, CDKN2A, PTCH1, PIK3CA	Cell cycle, NOTCH, PIK3-AKT, MAPK, DNA repair, RTKs signaling pathways	Elucidation the major cancer-related genes	(Munari et al., 2021)
ESCC	WES	Tissue	WES (n = 28)	TP53, TTN, CSMD3, SYNE1, LRP1B, PIK3CA, DNAH5, NOTCH1	PIK3CA-AKT pathway	PIK3CA serve as potential therapeutic target	(Mangalaparthi et al., 2020)
ESCC	WES	Tissue	WES (n = 10)	TP53, NCOR1, APC, KMT2C, CDKN1B, NOTCH, MYC, PIK3CA, SOX2, CCND1, SHANK2, CTTN, KRAS, SBS16, SBS26	RTK, homeostasis, EMT, invasion, metastasis, cell cycle, checkpoint, chromatin remodel pathways	SBS16 increase DNA damage, SBS26 defects in mismatch repair and microsatellite instability	(Erkizan et al., 2021)
ESCC	NGS	Tissue	NGS (n = 148)	CDKN2A, FGFR3, IKZF1, BCL11B, NKX2-1, SMARCB1	NA	CDKN2A might be important in ESCC development	(Onozato et al., 2021)
ESCC	WES, TCR-seq	Tissue, blood	WES (n = 185), TCR-seq (n = 31)	TP53, NFE2L2, BRIP1, BRCA1/2, NOTCH1, ZNF750, AXIN2, KMT2D, EP300, KDM6A, SMARCA4, CREBBP, PIK3CA, ERBB4, MET, MTOR, FBXW7, PTCH1, CD274	Cell cycle, cell differentiation, histone modification, RTK-RAS-PI3K, cell proliferation pathway	ERBB4 serve as oncogenic gene, BRCA1/2 serve as therapeutic target	(Yan et al., 2019)
ESCC	WGS	Tissue	WGS (n = 6)	H3K9me3, H3K27me3, EZH2/SUZ12, lncRNA ESCCAL-1	WNT2/ β -catenin pathway	DNA methylation gain at the promoter region activates the WNT2/ β -catenin pathway	(Cao et al., 2020)
ESCC	WGS	Tissue, blood	WGS (n = 94)	CDKN2A, TP53, NOTCH1, FBXW7, NFE2L2, AJUBA, LRP1B, TTC28	RTK/RAS/PI3K, cell cycle, WNT, Notch, TP53 pathway, TNF pathway	LRP1B and TTC28 are the most frequently affected genes by structural variation	(Chang et al., 2017)

(Continued)

Table 1. Continued.

Caner type	Platform	Sample sources	Number of samples	Key mutant genes	Key mutant pathways	Function	References
ESCC	WES	Tissue	WES (n=88)	TP53, NOTCH1, PIK3CA, NFE2L2	RTK/PI3K, NOTCH family, and NFE2L2 pathways	Altered gene regulation of imprinted genes and FAT family genes were revealed	(Takemoto et al., 2021)
ESCC	WGS	Tissue, blood	WGS (n=4)	KISS1R, AMH, MNX1, WNK2, PRKRIR, MACROD2, FHIT, PARK2	NA	MACROD2, FHIT and PARK2 often intragenic deletions	(Hu et al., 2016)
ESCC, EAC	WGS, SNP array	Tissue	WGS (n=51), SNP array (n=113)	CCND1, SOX2, TP63, ERBB2, VEGFA, GATA4, GATA6	RTK/RAS/PI3K, cell cycle, cell differentiation, chromatin remodeling	Three molecular subclasses were identified	(Cancer Genome Atlas Research Network et al., 2017)
ECRT	WES	Tissue	WGS (n=42)	TGFB2, IRF2BPL, PIK3CA, NFE2L2	DNA-repair, NOTCH family, Hippo pathway, Histone-modifying gene, RTK-RAS pathway, PI3K pathway, cell cycle, Nrf2 pathway	Predict prognosis	(Li et al., 2022)
ESCC	WES, SNP array	Tissue	WES (n=144), SNP array (n=144)	TP53, CCND1, CDKN2A, FBXW7, MLL2, EP300, CREBBP, TET2, NOTCH1, NOTCH3, FAT1, YAP1, AJUBA, PIK3CA, EGFR, ERBB2, ADH1B, ALDH2, CYP2A6	Cell cycle, epigenetic processes, the NOTCH, WNT and receptor-tyrosine kinase-phosphoinositide 3-kinase signaling pathways	EP300 and TET2 correlated with survival times, ZNF750 associated with the APOBEC signature	(Sawada et al., 2016)
ESCC	WES	Tissue	WES (n=302)	TP53, NOTCH1, FAT1, ZNF750, RB1, PTCH1, PIK3CA, FBXW7, NFE2L2, CDKN2A, FAM90A1, TNRC6A, ZNF750, CDC27	NA	ZNF750 and CDC27 are major candidate driver genes	(Guo et al., 2018)
ESCC	WGS	Tissue	WGS (n=52)	CDKN2A, FHIT, KDM6A, RB1, FAT, NFE2L2, ERBB4, CUL3, PTEN, ZNF750, NOTCH1, MYC, PTHLH	NA	PTHLH act as an oncogene	(Cui et al., 2022)
ESCC	WES	Tissue	WES (n=186)	TP53, NOTCH3, PTPRC	DNA mismatch repair	Immune microenvironment shapes the tumor evolution and promotes immune evasion of the tumor	(Cui et al., 2023)
ESCC	WES	Tissue	WES (n=102)	TP53, MUC16, FAT3, SYNE1, AKAP9, FAT4, AKAP9, MCAF1, PGK1	DNA replication, cell cycle, ECM signaling	SBS16 associated with alcohol drinking, promoted DNA replication, APOBEC signature was overrepresented in the nonsmoking/nondrinking patients	(Li et al., 2023b)
ESCC	WES	Tissue, blood	WES (n=125)	TP53, PIK3CA, FAT1, ADGB, DOPEY1, HECW1, LAMA1, THSD7A, USP9Y, PRKAG2, PLCH2, RBPJ, NPIPA5, MIB2	TGFβ signaling, WNT signaling	FAT1, ADGB, DOPEY1, HECW1, LAMA1, THSD7A, USP9Y, PRKAG2, PLCH2, NPIPA5 and MIB2 serve as potential target, CCND1 amplification promoted cell proliferation	(Liu et al., 2020b)

(Continued)

Table 1. Continued.

Caner type	Platform	Sample sources	Number of samples	Key mutant genes	Key mutant pathways	Function	References
ESCC	WES	Tissue	WES (n=64)	PIK3CA, NFE2L2, MTOR, TP53, KMT2D, ZNF750, ERBB4, FGFR2, BRCA2, ATM	PI3K/MTOR pathway	ERBB4, FGFR2, BRCA2, ATM, TP53 act as candidate actionable targets	(Hao et al., 2016)
ESCC	WGS, WES	Tissue	WGS (n=10), WES (n=57)	VANG1, SHANK2, MYBL2, FADD, MYBL2	Wnt/ β -catenin pathway	MYBL2 promotes proliferation and metastasis	(Qin et al., 2016)
ESCC	WGS, WES	Tissue	WGS (n=14), WES (n=90)	PIK3CA, CBX4, CBX8, AJUBA, ZNF750, PTCH1, SOX2	NOTCH signaling, PI3K pathway, p53 pathway, Hedgehog signaling, MAPK pathway, chromatin remodeling, and regulation of cell adhesion	AJUBA and ZNF750 act as tumor suppressors, SOX2 is the most likely target	(Zhang et al., 2015b)
ESCC	WGS	Tissue	WGS (n=155)	TP53, CDKN2A/B, NFE2L2, CCND1, CTTN, ANO1, ORAOV1, SOX2, NEUROG1, RUNX3	Cell cycle, NRF2 and SOX2 signaling	Identified potential targets for each subtype	(Liu et al., 2023b)
ESCC	WES	Tissue	WES (n=56)	TP53, KMT2D, ZNF750, IRF5, KMT2D, TET2, KAT2A	NA	ZNF750 as a metastasis suppressor, TP53 missense mutations associated with poor overall survival	(Dai et al., 2017)

NA, not provided by the author.

have an approximately fourfold increased ESCC risk compared with noncarriers (Wu et al., 2012), while tobacco chewers exhibited a higher frequency of C: G>A: T transpositions compared with smokers and nontobacco users in India (Mangalaparthy et al., 2020). CDKN2A mutations were enriched in nonsmoking, nondrinking female patients, suggesting alternative carcinogenic pathways (Onozato et al., 2021).

Beyond risk factors, specific mutations also drive metastatic progression. Analysis of paired tumors from patients with esophageal and lung squamous carcinomas revealed clonal relatedness in some cases, indicating metastatic spread, whereas others appeared genetically independent (Xue et al., 2020). WES analysis of primary tumors and metastatic lymph nodes identified recurrent mutations in TP53, KMT2D, ZNF750, and IRF5—particularly the loss-of-function mutations in ZNF750, which may serve as a metastasis suppressor. Mutations in epigenetic regulators (KMT2D, TET2, KAT2A) and deletions of histone variant genes (6p22, 11q23) suggest a shift toward a more plastic, invasive chromatin state during metastasis (Dai et al., 2017).

Genomic alterations contribute to chemoradiotherapy resistance

Intrinsic and acquired resistance to chemoradiotherapy is strongly linked to specific genomic defects. MYC amplification correlates with poor survival and persistent radiotherapy resistance, and MYC knockdown resensitizes ESCC cells (Hirata et al., 2021). Additional mechanisms include SLC7A8 mutations driving multidrug resistance and promoter hypomethylation of genes such as SLC8A3, associated with resistance phenotypes and enriched in protein digestion/absorption pathways (Min et al., 2021). These findings support the development of genomically guided patient stratification for combined-modality therapies.

Genomic distinctions between ESCC and EAC

Although ESCC and EAC are both forms of esophageal cancer, their genomic architectures are highly divergent. ESCC exhibits more frequent CNA amplifications in CDKN2A, EGFR, KRAS, MYC, CDK6, MET, and particularly squamous lineage drivers (CCND1, SOX2, TP63), whereas EAC harbors a different mutational burden (Bandla et al., 2012). NOTCH1 mutations are present in 21% of ESCC but absent in EAC; notably, their prevalence is higher in North American ESCC compared with Chinese cohorts (Agrawal et al., 2012). WGS studies further show shared alterations in classic cancer genes (TP53, BRCA2, ERBB2, FBXW7, PTCH, NF1) but also identify ESCC-specific loci such as KISS1R, AMH, MNX1, WNK2, and PRKRIR, as well as recurrent intragenic deletions in MACROD2, FHIT, and PARK (Hu et al., 2016). These distinctions highlight the necessity of subtype-specific diagnostic markers and therapeutic strategies.

Epigenetic dysregulation contributes to ESCC pathogenesis

Epigenomics investigates genome-wide epigenetic modifications, including DNA methylation, histone modifications, and chromatin remodeling, which regulate gene expression without altering the underlying DNA sequence. Aberrant epigenetic regulation and the resulting transcriptional reprogramming are recognized hallmarks of cancer (Kanwal and Gupta, 2012; Lima et al., 2011; Ma et al., 2016). Although early ESCC studies largely relied on candidate gene approaches, recent multi-omics investigations have provided a more comprehensive view of the ESCC epigenome, revealing dysregulated methylation, enhancer remodeling, and chromatin accessibility patterns that collectively reshape transcriptional programs during tumorigenesis.

Aberrant DNA methylation leads to gene silencing

DNA methylation remains the most extensively characterized epigenetic alteration in ESCC. A multinational cohort analyzed with the MethylationEPIC array identified tumor-specific methylation changes and highlighted PAX9, SIM2, and THSD4 as diagnostic candidates (Talukdar et al., 2021). Multi-omics profiling further demonstrated that 98% of CpG loci in ESCC are globally hypomethylated, whereas hypermethylation is enriched at polycomb-regulated regions bound by EZH2/SUZ12 (Cao et al., 2020). Additional genome-wide studies reported large numbers of differentially methylated CpG sites and constructed diagnostic or prognostic methylation signatures, including 12-CpG and 4-CpG panels (Xi et al., 2022a), as well as other methylation-based prognostic models (Chen et al., 2020b). Circulating methylation markers such as EPB41L3, GPX3, and COL14A1 offer promise for noninvasive detection (Li et al., 2014c). In a Northeast Indian cohort, genome-wide methylation profiling using the Infinium 450k array identified distinct patterns of hypermethylated and hypomethylated genes, and several corresponding circulating proteins, including IL22RA2, TNFSF13B, SERPINA4, and TAC3, were validated in serum, indicating their potential as noninvasive diagnostic biomarkers (Singh et al., 2015).

Public datasets have expanded methylation profiling efforts, revealing LINE-1 hypomethylation and promoter hypermethylation of classical tumor suppressors including RASSF1, p16INK4a, RUNX3, and APC (Hoshimoto et al., 2015). Integrative expression-methylation analyses (GSE51287 and GSE26886) further identified IL-6, MMP3, MMP9, and SPP1 as potential biomarkers (Peng et al., 2019; Takahashi et al., 2013). TCGA-derived analyses identified methylation-driven prognostic genes such as ABCD1, SLC5A10, and ZNF69 (Lu et al., 2019), and additional survival models have been proposed, including m6A-related signatures (Pu et al., 2022) and population-specific CpG panels (Han et al., 2020; Li et al., 2019b).

Histone modifications disrupt transcriptional regulation

Although fewer studies have profiled histone marks in ESCC, key regulatory patterns have emerged. Overexpression of EZH2, the H3K27me3 methyltransferase, leads to silencing of tumor suppressor genes and correlates with poor prognosis (Chen et al., 2011). HDAC1 and HDAC2 upregulation promotes proliferative transcriptional programs (Toh et al., 2013). Integrative analyses also reveal enrichment of active histone marks (H3K4me3, H3K27ac) at promoters and enhancers that regulate pathways such as cell cycle progression and EMT, underscoring the contribution of histone regulators to ESCC transcriptional dysregulation (Yi et al., 2020).

Chromatin remodeling reprograms the ESCC epigenome

Dysregulation of chromatin remodeling complexes, particularly SWI/SNF components such as ARID1A, SMARCA4, and PBRM1, is frequently observed in ESCC and contributes to widespread changes in chromatin accessibility (Nakazato et al., 2016). Loss of ARID1A impairs DNA repair and promotes transcriptional silencing of differentiation-associated genes (Kearns et al., 2025). Integrated ATAC-seq and RNA-seq analyses further reveal ESCC-specific enhancer and super-enhancer activation patterns that drive oncogene expression. Cooperative dysregulation between remodelers and histone modifiers, such as ARID1A loss with EZH2 overactivity, can reinforce stem-like transcriptional programs and promote aggressive tumor phenotypes. These findings highlight chromatin remodeling as a central mechanism in ESCC epigenetic reprogramming and a potential therapeutic vulnerability.

Transcriptomic alterations in ESCC progression

The transcriptome, unlike the relatively stable genome, reflects the dynamic state of cellular activity across tissues and disease stages. In ESCC, transcriptomic profiling has been widely used to identify dysregulated genes and pathways that drive tumor initiation, progression, and treatment response. Microarray-based approaches and RNA sequencing (RNA-seq) have together generated a large body of data, enabling the discovery of molecular subtypes, prognostic signatures, and potential therapeutic targets.

Microarray and RNA-seq reveal transcriptional landscapes

Microarray technology has been extensively applied in ESCC to screen gene expression changes and derive prognostic markers. For example, integration of exome sequencing from 81 tumor-normal pairs with microarray data from 119 patients (GSE53624) identified ANO1 and

MMP3 as prognostic genes associated with outcome (Yu et al., 2020). Similarly, RNA-seq analysis of 179 ESCC patients (GSE53625) revealed PLEK2 as a poor prognostic indicator that promotes metastasis and chemoresistance via LCN2 upregulation (Wang et al., 2021a). Other microarray-based analyses (GSE44021, GSE121931, and GSE23400) linked survival to multi-gene expression signatures involving NF1, ASXL1, HSPA4, and additional candidates (Chen et al., 2020b; Su et al., 2011; Yang et al., 2020a). Although many cohorts are relatively small, these datasets, now archived in Gene Expression Omnibus (GEO), together provide a valuable resource for meta-analysis and biomarker validation (Fig. 2 and Table 2).

RNA-seq has further enabled high-resolution characterization of ESCC transcriptomes. Studies have identified differentially expressed genes, molecular subtypes, metabolic perturbations, immune features, and widespread alternative splicing events. For example, RNA-seq of tumor and matched normal tissues revealed distinct expression patterns among CLIC family members (Liu et al., 2020a), while integration of RNA-seq with targeted metabolomics highlighted dysregulated tyrosine metabolism and associated diagnostic metabolites in primary and metastatic ESCC (Cheng et al., 2021). Immunogenomic analyses using B-cell receptor libraries from 119 tumors and adjacent tissues showed that BCR subtypes correlate with prognosis and immune contexture (Wang et al., 2022d). Subtype classification of 125 tumors identified three ESCC subgroups characterized by differences in metabolic, inflammatory, and proliferative programs, with CCND1 overexpression linked to enhanced proliferation (Liu et al., 2020b). Alternative splicing analyses identified SF3B4 as an overexpressed splicing factor regulating numerous targets and representing a potential therapeutic node (Ding et al., 2021). Additional work has functionally validated transcriptional regulators such as ZNF750 and BRAP as prognostic factors with roles in squamous differentiation, invasion, NF- κ B signaling, and lymphangiogenesis (Bi et al., 2020; Zhao et al., 2017). Together, these RNA-based studies delineate a complex transcriptional landscape in ESCC and nominate multiple candidate biomarkers and pathways for clinical translation. Additional RNA-seq-based studies using clinical ESCC samples are summarized in Table 2.

Dysregulation of ncRNAs in ESCC

ncRNAs, including long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs), are key regulators of gene expression and have emerged as critical players in ESCC biology. High-throughput profiling has revealed that multiple classes of ncRNAs participate in cell cycle control, apoptosis, invasion, metastasis, immune regulation, and therapy response (Table 3).

lncRNAs regulate tumor biology and serve as biomarkers

lncRNAs, defined as transcripts over 200 nucleotides with minimal or no protein-coding capacity (Bazin et al., 2017), are widely dysregulated in ESCC. Numerous studies using GEO and TCGA datasets have constructed lncRNA-based prognostic signatures. Examples include LINC00551 as a candidate prognostic marker (Peng et al., 2021), an eight-lncRNA panel from GSE53625 (Li et al., 2020c), three-lncRNA and seven-lncRNA signatures that predicted overall and disease-free survival (Huang et al., 2018; Mao et al., 2018), as well as additional models derived from GSE92986, GSE53625 and other cohorts (Cao et al., 2022; Li et al., 2014a; Weng et al., 2020; Yu et al., 2019b). These signatures consistently support the prognostic value of lncRNA expression patterns.

Mechanistic studies have shown that individual lncRNAs modulate key oncogenic pathways. LINC00680 acts as a ceRNA for miR-423-5p to regulate PAK6 and promote growth (Xue et al., 2022); HERES and CASC9 interact with EZH2 to modulate Wnt signaling and repress PDCD4, respectively (Wu et al., 2017; You et al., 2019). Other lncRNAs such as Lnc-KIAA1244-2, TN-AS1, and LINC00491 regulate proliferation and apoptosis (Ma et al., 2019; Ye et al., 2021a), while HCG22 suppresses migration via SPINK7/ADAMTS12 (Li et al., 2020d). CASC9 and DUXAP8, identified from multiple public datasets, are associated with metastatic phenotypes (Liu et al., 2018; Pan et al., 2016). Expression profiling in small tissue cohorts further expanded the catalog of cancer-related lncRNAs and mRNAs in ESCC (Wang et al., 2018; Yao et al., 2016).

Recent integrative analyses have linked lncRNAs to immune regulation, autophagy and ferroptosis. An eight-lncRNA immune-related signature was associated with immune checkpoint response (Zhu et al., 2021b); autophagy-related and ferroptosis/iron-metabolism-related lncRNAs were connected to survival and risk stratification (Niu et al., 2022; Zhao et al., 2022d). m⁷G-related lncRNAs and immune-associated multi-lncRNA signatures built from TCGA and GEO datasets were shown to predict prognosis and potential response to immunotherapy (Pang et al., 2021; Zhao et al., 2022a).

lncRNAs also show promise as noninvasive biomarkers. A five-lncRNA panel in plasma exosomes demonstrated diagnostic potential (Jiao et al., 2020), while LINC00324 and LOC100507053 were detected in peripheral blood and proposed as liquid biopsy markers (Sharma et al., 2022). Additional prognostic and functional lncRNAs include LINC01088, which suppresses growth through the NPM1-HDM2-p53 axis (Liang et al., 2023), LINC00022, which promotes tumorigenesis via FTO-mediated m⁶A demethylation (Cui et al., 2021), and LINC01234, which inhibits ESCC progression through miR-193a-5p/CCNE1 regulation (Ma et al., 2020). Network-based analyses have further revealed lncRNA-mRNA regulatory axes and exosome-related

lncRNA pairs associated with immune infiltration, microbiota composition, and clinical outcomes (Alaei et al., 2019; Zhao et al., 2022b). Collectively, these findings underscore lncRNAs as versatile regulators with strong potential for biomarker development and therapeutic stratification. A summary of key lncRNA studies is presented in Table 3 and Fig. 2.

miRNAs act as posttranscriptional regulators in progression

miRNAs are small ncRNAs, typically 20 to 22 nucleotides in length, that fine-tune gene expression posttranscriptionally, and play essential roles in cell proliferation, differentiation, apoptosis, and tumor progression. In ESCC, aberrant miRNA expression has been extensively studied for its diagnostic, prognostic, and therapeutic relevance (Table 3).

Multiple studies have proposed miRNA signatures with strong predictive power. Salivary EV-derived miRNA panels (e.g., miR-1268a, miR-4505, miR-1972, miR-4274, miR-4701-3p, miR-6126) showed potential for early detection (Li et al., 2023a). Expression of hsa-miR-103/107 was associated with poor survival (Guo et al., 2008), whereas miR-30e, miR-124, and miR-574-3p correlated with favorable prognosis or postoperative outcomes (Okumura et al., 2016; Yang et al., 2020a). A four-miRNA model based on GSE43732 predicted survival (Chen et al., 2014), and serum miRNAs such as miR-25, miR-100 and miR-1246 were linked to prognosis and served as circulating biomarkers (Takeshita et al., 2013; Wu et al., 2014a).

Public databases have facilitated the construction of more complex models, including six-miRNA and eight-miRNA signatures derived from GSE43732, TCGA, GSE55856 and other datasets that stratify risk and distinguish early ESCC from healthy controls (Li et al., 2019a; Mao et al., 2019; Miyoshi et al., 2022). Additional panels and serum miRNA sets were associated with disease stage, recurrence, and survival (He et al., 2019; Sun et al., 2018; Wang et al., 2017; Yu et al., 2019c; Zhang et al., 2010). A five-miRNA set (hsa-mir-103-1, hsa-mir-18a, hsa-mir-324, hsa-mir-369, and hsa-mir-320b-2) derived from TCGA showed diagnostic and preventive potential and correlated with survival (Zhao et al., 2015).

Functionally, numerous miRNAs have been implicated in ESCC pathogenesis. Diagnostic serum panels (e.g., miR-8073, miR-6820-5p, miR-6794-5p, miR-3196, miR-744-5p, miR-6799-5p) demonstrated high discriminative power (Sudo et al., 2019), and miRNA-based tests outperformed conventional markers like CEA and CA19-9 in distinguishing ESCC and ESD from healthy controls (Shen et al., 2019). Specific miRNAs such as miR-21-5p, miR-146b-5p, and miR-210-3p were altered in superficial ESCC (Fujihara et al., 2021), while plasma miR-25 and other circulating miRNAs tracked tumor dynamics and diagnosis (Ibuki et al., 2020; Komatsu et al., 2014;

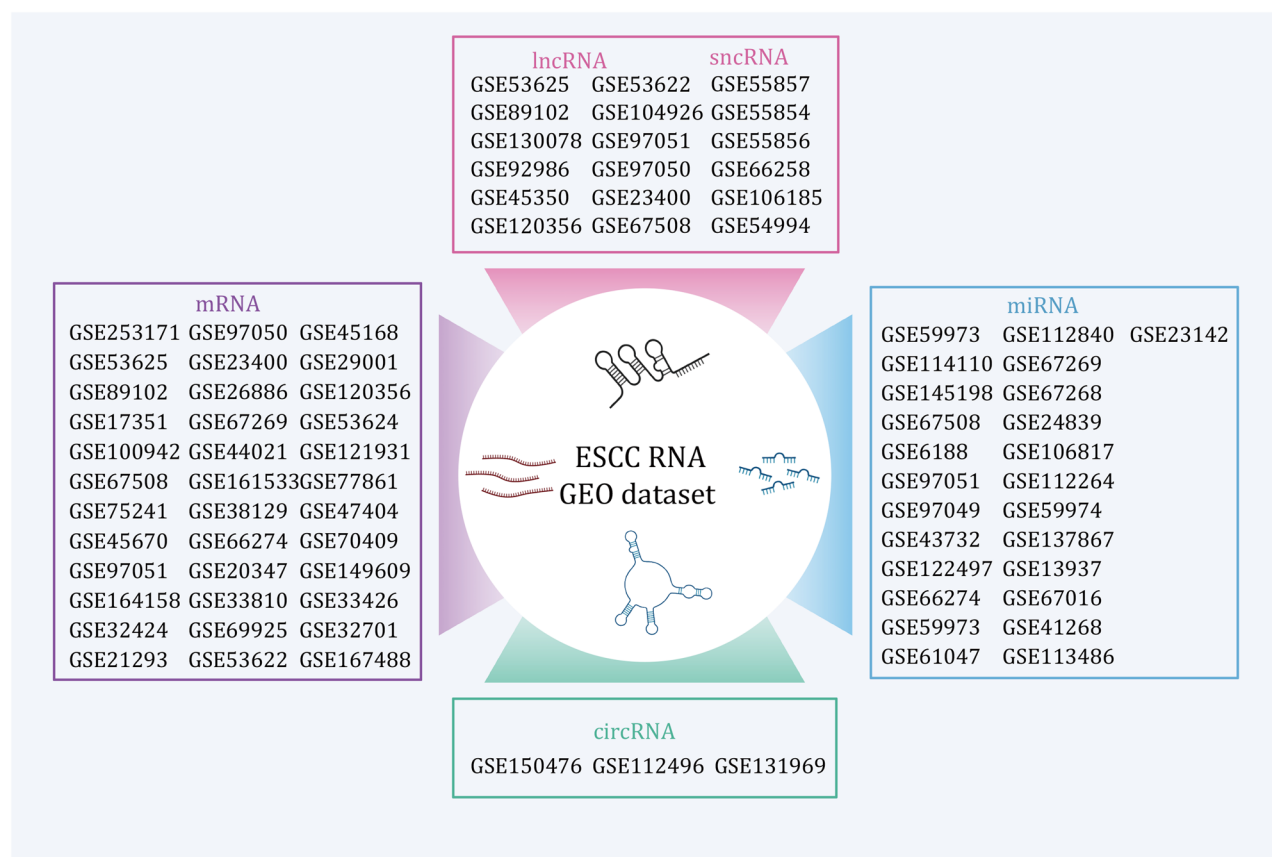


Figure 2. Public GEO transcriptomic datasets for ESCC. This figure summarizes Gene Expression Omnibus (GEO) datasets generated from ESCC samples, covering multiple RNA layers, including mRNA, lncRNA, sncRNA, miRNA, and circRNA.

Zheng et al., 2018). A four-miRNA model (GSE122497, GSE106817, and GSE112264) demonstrated diagnostic accuracy (Song et al., 2021).

Several miRNAs directly regulate oncogenic pathways and may be exploited therapeutically. miR-130b promotes tumorigenesis via PTEN/Akt signaling (Yu et al., 2015; Zhu et al., 2019a), whereas miR-375 and miR-99a suppress proliferation, metastasis, and IGF1R signaling (Kong et al., 2012; Mei et al., 2017a; Yi et al., 2017). Downregulation of miR-126, miR-143, miR-145, and other tumor-suppressive miRNAs contributes to increased motility and EMT (Liu et al., 2015; Mei et al., 2017b; Wu et al., 2011a; Yang et al., 2019). Additional work has identified apoptosis-related miRNAs (e.g., miR-202 via HSF2 targeting), key regulators from network analyses (miR-181a, miR-202, miR-155), and oncogenic miR-21 driving RASA1 downregulation and EMT (Chen et al., 2019; Meng et al., 2017; Yang et al., 2015). In addition, miR-1 functions as a tumor suppressor (Yao et al., 2015). MicroRNA-384, selected from GSE17351, GSE29001, and GSE45168, suppressed tumor growth by inhibiting LIMK1/cofilin signaling (Yu et al., 2019a).

In addition, a comparative analysis of lncRNA, miRNA, and mRNA expression across normal epithelium, low-grade intraepithelial neoplasia (LGIN), high-grade intraepithelial neoplasia (HGIN), and carcinoma tissues revealed coordinated transcriptomic alterations during

ESCC development, particularly during the transition from HGIN to invasive cancer. These findings suggest that different RNA classes participate in shared regulatory programs underlying esophageal tumorigenesis (Li et al., 2014b).

circRNAs emerge as novel players in ESCC

CircRNAs are covalently closed ncRNAs lacking a 5' cap or 3' tails (Kristensen et al., 2019). In ESCC, circRNAs are increasingly recognized for their diagnostic and mechanistic importance. Early circRNA microarray profiling identified multiple dysregulated circRNAs with biomarker potential (Shi et al., 2018). Plasma circRNA profiling revealed hsa_circ_0004771 as a promoter of ESCC progression via the miR-339-5p/CDC25A axis and a candidate noninvasive biomarker. CircCNTNAP3, circCYP24A1, circRUNX1, and circARAP2 have been shown to regulate proliferation, metastasis, and inflammatory signaling through TP53, NF- κ B, FOXP3, and FOXM1-related pathways (Gu et al., 2022; Wang et al., 2020a, 2022a; Xu et al., 2022). Conversely, hsa_circ_0001946 exhibits tumor-suppressive properties and predicts recurrence and survival (Fan et al., 2019), while LPAR3 circRNA promotes migration and metastasis via miR-198 sponging (Shi et al., 2020).

Using public datasets, additional functional circRNAs have been identified, including circBCAR3 (hsa_circ_0007624),

Table 2. Representation of transcriptomics studies in ESCC.

Cancer type	Dataset	Platform	Sample sources	Number of samples	RNA	Key molecular	Function	References
ESCC	GSE149609	GPL20301	Tissue	n= 10	mrNA	WNT2	WNT2 activate the WNT pathway	(Cao et al., 2020)
ESC	NA	Illumina HiSeq2000	Tissue	n= 57	mrNA	FAT1, FAT2	FAT1 and FAT2 serve as tumor suppressor genes	(Takemoto et al., 2021)
ESC	EGAS00001003832	Illumina HiSeq X Ten	Tissue	n= 186	mrNA	HLA-A, HLA-B, B2M	HLA-A, HLA-B, and B2M relate to immune infiltration	(Cut et al., 2023)
ESCC	HRA000178	Illumina HiSeq X Ten	Tissue	n= 125	mrNA	CGND1	CGND1 associated with poor prognosis	(Liu et al., 2020b)
ESCC	HRA003107	Illumina HiSeq X Ten	Tissue	n= 155	mrNA	CGND1, CDKN2A, NFE2L2, SOX2, ERBB2	CGND1/CDKN2A related with cell cycle, NFE2L2 related with antioxidant stress response, SOX2 related to transcriptional activation, ERBB2 act as oncogenic signaling	(Liu et al., 2023b)
ESCC	GSE44021	GPL571	Tissue	n= 113	mrNA	MMP1, SPP1, COL11A1, COL1A1, POSTN, MMP12, MAGEA6, MAGEA3, COL1A2, KRT17, CRISP3, CRNN, MAL, TGM3, CLCA4, SCEL, CRCT1, SLURP1, TMPPRS11E, FLG	Eight genes (NFI, ASXL1, HSPA4, TGOLN2, BAIAP2, EZH2, CHAF1A, SUPT7L) were associated with survival	(Yang et al., 2020a)
ESCC	GSE23400	GPL96	Tissue	n= 53	mrNA	COL1A2, COL3A1, MET, KRT14, SPINK7/ECG2, HPGD	COL1A1 and COL3A1 related with prognosis	(Su et al., 2011)
ESCC	NA	Illumina	Tissue	n= 10	mrNA	CLIC3, CLIC4	Low level of CLIC3 and high level of CLIC4 act as prognostic biomarkers	(Liu et al., 2020a)
ESCC	PRJNA298963	Illumina HiSeq 2500	Tissue	n= 15	mrNA	IL4I1, MAOA, ALDH3A1, ALDH1A3, ALDH3B1, ALDH3B2	Related with tumorigenesis and metastasis	(Cheng et al., 2021)
ESCC	PRJCA001577	Illumina X Ten	Tissue	n= 119	mrNA	IGH, CD138, MS4A1, IGHV3-74	Related with immunotherapy targeting B cells	(Wang et al., 2022d)
ESCC	GSE53624	GPL18109	Tissue	n= 119	mrNA	ENST00000435885.1, XLOC_013014, ENST00000547963.1	Three-lncRNA signature act as an independent prognostic factor	(Li et al., 2014a)
ESCC	GSE53625	GPL18109	Tissue	n= 179	mrNA	ENST00000435885.1, XLOC_013014, ENST00000547963.1	Three-lncRNA signature act as an independent prognostic factor	(Li et al., 2014a)
ESCC	GSE53622	GPL18109	Tissue	n= 60	mrNA	ENST00000435885.1, XLOC_013014, ENST00000547963.1	Three-lncRNA signature act as an independent prognostic factor	(Li et al., 2014a)
ESCC	GSE121931	GPL15207	Tissue	n= 125	mrNA	CGND1, CSF3R, E2F2, JUP, RARA and TCF7	Signature can predict the clinical outcome	(Li et al., 2020b)
ESCC	GSE20347	GPL571	Tissue	n= 17	mrNA	NA	CNNLOH (genomic instability) linked to abnormal gene expression	(Hu et al., 2010)
ESCC	GSE17351	GPL570	Tissue	n= 5	mrNA	PTGES	PTGES act as a novel target of hypoxia	(Lee et al., 2010)
ESCC	GSE38129	GPL571	Tissue	n= 30	mrNA	NA	Biallelic loss is significantly associated with reduced mRNA expression	(Hu et al., 2015)
ESCC	GSE77861	GPL570	Tissue	n= 7	mrNA	NFE2L2	NFE2L2 regulator of detoxification/oxidative-stress defense	(Erkizan et al., 2017)
ESCC	GSE47404	GPL6480	Tissue	n= 71	mrNA	GRB7	GRB7 act as a potential new therapeutic target	(Sawada et al., 2015)
ESCC	GSE29001	GPL571	Tissue	n= 12	mrNA	ODC1, POSTN, ASPA	ODC1, POSTN, and ASPA identified as therapeutic targets	(Yan et al., 2012)

(Continued)

Table 2. Continued.

Cancer type	Dataset	Platform	Sample sources	Number of samples	RNA	Key molecular	Function	References
ESCC	GSE33426	GPL571	Tissue	n=9	mRNA	NA	3D tumor profiling reveals ESCC contains little regional mRNA heterogeneity	(Yan et al., 2013)
ESCC	GSE70409	GPL13287	Tissue	n=17	mRNA	MMP1	MMP1 is a potential noninvasive biomarker for detection and prognosis	(Chen et al., 2016)
ESCC	GSE75241	GPL5175	Tissue	n=15	mRNA	FOXM1	PIK3R3 is a novel FOXM1 target	(Nicolau-Neto et al., 2018)
ESCC	GSE161533	GPL570	Tissue	n=28	mRNA	COL1A1, SPARC, FN1	Associated with ESCC tumor status	(Shen et al., 2021b)
ESCC	GSE164158	GPL20795	Tissue	n=8	mRNA	PTTG1	PTTG1 act as a new target	(Chen et al., 2020a)
ESCC	GSE26886	GPL570	Tissue	n=10	mRNA	WDR66	WDR66 act as biomarker for risk stratification and drug target	(Wang et al., 2013)
ESCC	GSE100942	GPL570	Tissue	n=4	mRNA	RHCG	RHCG act as a transcription regulator	(Ming et al., 2018)
ESCC	GSE33810	GPL570	Tissue	n=2	mRNA	CRNN	CRNN act as a tumor suppressor gene	(Chen et al., 2013)
ESCC	GSE32424	GPL10999	Tissue	n=7	mRNA	Rab25	Rab25 act as a prognostic biomarker and a novel therapeutic target	(Tong et al., 2012)
ESCC	GSE45168	GPL13497	Tissue	n=5	mRNA	NEK6	NEK6 serve as an independent prognostic indicator	(Li et al., 2020a)
ESCC	GSE69925	GPL570	Tissue	n=274	mRNA	PRF1, GZMB, IFNG, CASP1, CASP4, TNFSF4, TNFSE11, TNFSF13B, TNFAIP8	Related with immune	(Tanaka et al., 2015)
ESCC	GSE45670	GPL570	Tissue	n=28	mRNA	MMP1, LIMCH1, C1orf226	Related with chemoradiotherapy	(Wen et al., 2014)
ESCC	GSE32701	GPL570	Tissue	n=20	mRNA	ZEB1, ZEB2, TWIST1	Related with epithelial-mesenchymal transition	(Aoyagi et al., 2011)
ESCC	GSE21293	GPL570	Tissue	n=35	mRNA	POSTN	Related with invasion	(Michaylira et al., 2010)
ESCC	PRJCA000354	Illumina HiSeq X Ten	Tissue	n=94	mRNA	CUL3, RBPJ	Altered the malignant phenotypes	(Chang et al., 2017)
ESCC	NA	Illumina HiSeq2000	Tissue	n=59	mRNA	TP63, KRT5/15, BNC1, DSC3, DSG3	Act as diagnosis markers	(Liu et al., 2016)
ESCC	OEP002359	NA	Tissue	n=24	mRNA	FBL	FBL predicts unfavorable patient survival	(Jin et al., 2021)
ESCC	GSE167488	GPL24676	Tissue	n=3	mRNA	NA	NA	NA
ESCC	TCGA	NA	Tissue	n=95	mRNA	CCND1, SOX2, TP63, ERBB2, VEGFA, GATA4, GATA6	RTK/RAS/PI3K, Cell cycle, Cell differentiation, Chromatin remodeling	(Cancer Genome Atlas Research Network et al., 2017)
ESCC	HRA000021	NA	Tissue	n=97	mRNA	ZNF750	Act as a metastatic and prognostic biomarker, and therapeutic target	(Bi et al., 2020)
ESCC	GSE253171	GPL30375	Tissue	n=3	mRNA	AMPK	Act as potential therapeutic targets	(Sun et al., 2025)
ESCC	GSE106185	GPL570	Tissue	n=23	mRNA	NA	NA	NA

NA, not provided by the author.

Table 3. Representation of ncRNA profiles in ESCC.

Cancer type	Dataset	Platform	Sample sources	Number of samples	RNA	Key molecular	Function	References
ESCC	GSE53625 (GSE53622 and GSE53624) GSE89102	GPL18109	Tissue	n = 358	LncRNA, mRNA	ENST00000435885.1, XLOC_013014, ENST00000547963.1 linc00460	Three-lncRNA signature act as an independent prognostic factor Act as biomarkers for diagnosis and treatment	(Li et al., 2014a)
ESCC	GSE67508	GPL16956	Tissue	n = 5	LncRNA, mRNA		Act as biomarkers for diagnosis and treatment	(Liang et al., 2017)
ESCC	GSE67508	GPL570	Cell	n = 2	mRNA, microRNAs	GBP1	GBP1 promotes lymph node metastasis	(Li et al., 2015)
ESCC	GSE97051 (GSE97049 and GSE97050)	GPL20115 GPL21572	Tissue	n = 7	lncRNA-miRNA-mRNA	lncRNA-TTN-AS1, miR-133b, FSCN1	Act as potential therapeutic targets	(Lin et al., 2018)
ESCC	GSE23400	GPL97 GPL96	Tissue	n = 5	LncRNA, mRNA	COL1A2, COL3A1, MET, KRT14, SPINK7/ECG2, HPGD	COL1A1 and COL3A1 related with prognosis	(Su et al., 2011)
ESCC	GSE67269 (GSE67268 and GSE 44021)	GPL19823	Tissue	n = 113	miRNA, mRNA	miR-30e, miR-124, NF1, ASXL1, HSPA4, TGOLN2, BAIAP2, EZH2, CHAF1A, SUPT7L	Associated with survival	(Yang et al., 2020a)
ESCC	GSE137867	GPL15207	Tissue	n = 4	microRNA, mRNA	hsa-miR-34b-3p, hsa-miR-127-5p, hsa-miR-144-3p, hsa-miR-486-5p, TNF, AKR1C1, AKR1C2, ICAM1, GPR68, GNB4, SERPINE1, MMP12	MMP12 might be a useful tumor biomarker and therapeutic target	(Wang et al., 2021c)
ESCC	GSE66274 GSE120356	GPL19823 GPL23178	Tissue Tissue	n = 30 n = 5	mRNA miRNA LncRNA, mRNA	NA Lnc-KIAA1244-2	NA Act as potent therapeutic target	(Hu et al., 2015) (Ma et al., 2019)
ESCC	GSE130078	GPL11154	Tissue	n = 23	LncRNA	HERES	Act as an early diagnostic and therapeutic target	(You et al., 2019)
ESCC	GSE92986	GPL22868	Tissue	n = 141	LncRNA	XLOC_007869, CK327190, XLOC_006476, ASLNC11164, BF894811, BQ376030, RP11-473M20.9	Associated with survival	(Weng et al., 2020)
ESCC	GSE45350	GPL13607	Tissue	n = 4	LncRNAs, mRNA	ESCCAL_1, HOTAIR	Act as potential diagnostic and prognostic biomarkers	(Cao et al., 2014)
ESCC	GSE104926	GPL21290	Tissue	n = 6	LncRNA, mRNA	RP5-1092A11.2	Associated with different disease status	(Tian et al., 2020)
ESCC	GSE55857 (GSE55854, GSE55856, GSE66258) GSE59973 GSE114110	GPL10558 GPL14613	Tissue	n = 108	snRNA	miR-223, miR-1269a	Act as prognostic marker	(Jang et al., 2017)
ESCC			Tissue	n = 3	miRNA	miR-195	Act as a tumor suppressor	(Fu et al., 2013)
ESCC			Tissue	n = 30	miRNA	miR-424	Act as a novel prognostic marker and an effective therapeutic target	(Wen et al., 2018)
ESCC	GSE145198 GSE6188	GPL16770 GPL24967	Tissue Tissue	n = 4 n = 31	miRNA microRNA	NA hsa-miR-103/107	NA Act as diagnostic and prognostic marker	NA (Guo et al., 2008)

(Continued)

Table 3. Continued.

Cancer type	Dataset	Platform	Sample sources	Number of samples	RNA	Key molecular	Function	References
ESCC	GSE43732	GPL16543	Tissue	n = 119	miRNA	hsa-miR-218-5p, hsa-miR-142-3p, hsa-miR-150-5p, hsa-miR-205-5p	Predict survival	(Chen et al., 2014)
ESCC	GSE122497	GPL21263	Serum	n = 566	microRNA	miR-8073, miR-6820-5p, miR-6794-5p, miR-3196, miR-744-5p, miR-6799-5p miR-16-5p, miR-451a, miR-574-5p	Act as diagnosis marker	(Sudo et al., 2019)
ESCC	GSE112840	GPL23365	Serum	n = 52	miRNA	NA	Act as biomarkers for diagnosis	(Zheng et al., 2018)
ESCC	GSE106817	GPL21263	Serum	n = 88	miRNA	NA	NA	(Yokoi et al., 2018)
ESCC	GSE112264	GPL21263	Serum	n = 50	miRNA	NA	NA	(Urabe et al., 2019a)
ESCC	GSE59974	GPL19007	Tissue	n = 17	miRNA	miR-145-5p, miR-152, miR-193b-3p, miR-376a-3p	Facilitate individualized treatment	(Wen et al., 2016)
ESCC	GSE61047	GPL17107	Tissue	n = 4	miRNA	microRNA-330-3p	Functions as an oncogene	(Meng et al., 2015)
ESCC	GSE13937	GPL8835	Tissue	n = 44	miRNA	miR-21, miR-181b, miR-155, miR-146b, miR-375, miR-203	miR-21 associated with prognosis	(Mathé et al., 2009)
ESCC	GSE67016	GPL19906	Cell line	n = 3	miRNA	miR-214-3p	Act as a tumor suppressor	(Phatak et al., 2016)
ESCC	GSE41268	GPL10850	Saliva	n = 7	miRNA	miR-10b*, miR-144, miR-451, miR-21	Act as distinctive miRNAs	(Xie et al., 2013)
ESCC	GSE113486	GPL21263	Serum	n = 40	miRNA	NA	NA	(Usuba et al., 2019)
ESCC	GSE23142	GPL7723	Tissue	n = 3	miRNA	miR-143, miR-145	Act as anti-oncomirs	(Wu et al., 2011a)
ESCC	E-MTAB-2208	NA	Tissue	NA	miRNA	miR-31	miR-31 act as a therapeutic target	(Iaccoli et al., 2015)
ESCC	GSE150476	GPL21825	Tissue	n = 3	circRNA	CircIMMP2L	Act as a biomarker for the diagnosis and prognosis	(Liang et al., 2021)
ESCC	GSE112496	GPL21825	Plasma	n = 5	circRNA	29 circRNAs, 2 miRNAs, and 10 hub genes	Help understand the circRNA-miRNA-mRNA regulatory mechanism	(Shen et al., 2021a)
ESCC	GSE131969	GPL19978	Tissue	n = 3	circRNA	circGSK3β	Serve as a novel diagnostic and prognostic biomarker	(Hu et al., 2019)

NA, not provided by the author.

hsa_circ_0006168, circRNA-DOPEY2, hsa_circ_0000277, and hsa_circ_0006948, which modulate processes such as metastasis and mTOR signaling (Liu et al., 2021b; Pan et al., 2019; Shi et al., 2019; Xi et al., 2022b; Zhou et al., 2021). The circ_0007624/miR-224-5p/CPEB3 axis suppresses tumor progression via EGFR/PI3K/AKT inactivation (Cheng et al., 2022). A four-circRNA signature (hsa_circ_0000005, hsa_circ_0007541, hsa_circ_0008199, and hsa_circ_0077536) has been proposed for risk stratification (Wang et al., 2021e), and circRNA-miRNA-mRNA networks derived from GSE112496 have begun to map the broader posttranscriptional landscape (Shen et al., 2021a).

RNA modifications alter gene function in ESCC

Emerging evidence has revealed that dysregulated epigenetic modifications, particularly RNA methylation, are critical drivers of tumor initiation, progression, and recurrence (Delaunay and Frye, 2019). Among these modifications, m⁵C is a conserved RNA modification present in rRNA, tRNA, lncRNA, and mRNA (David et al., 2017; Khoddami and Cairns, 2013; Squires et al., 2012). Transcriptome-wide profiling has shown globally increased m⁵C in ESCC, largely driven by overexpression of the methyltransferase NSUN2. Mechanistic experiments demonstrated that NSUN2 stabilizes GRB2 mRNA through an m⁵C-LIN28B-dependent pathway, thereby promoting ESCC initiation and progression (Su et al., 2021). These results highlight the oncogenic role of RNA methylation and suggest that targeting the m⁵C epitranscriptome could represent a novel therapeutic avenue.

Single-cell transcriptomics uncovers intratumoral heterogeneity

Single-cell transcriptomics has been extensively studied in ESCC, providing a powerful tool for dissecting tumor heterogeneity and the TME. By analyzing single-cell transcriptomes, researchers can investigate various aspects of the immune landscape of tumors, including immunosuppressive populations, immune cell subsets, and inter- and intratumoral heterogeneity (Fig. 3).

Early scRNA-seq studies analyzing paired ESCC tumors and adjacent nonmalignant tissues have established a comprehensive cellular atlas of the esophageal epithelium and tumor ecosystem. For example, profiling of ~128,000 cells from five paired samples revealed distinct malignant epithelial states marked by IGFBP2/IGFBP3, ODC1, and SOX4, in contrast to differentiation-associated markers such as CRCT1, CRNN, and SPINK5 in nonmalignant epithelium (Chen et al., 2021c). Importantly, malignant ESCC cells exhibited pronounced metabolic reprogramming, with oxidative phosphorylation contributing substantially to intratumoral metabolic heterogeneity. Transcriptional stratification further identified four malignant cell programs, among which a poorly differentiated state was strongly associated with survival, linking cellular state

heterogeneity to clinical outcome. Beyond tumor-intrinsic programs, single-cell analyses consistently demonstrate that ESCC is characterized by a profoundly immunosuppressive TME. Integration of scRNA-seq with TCR sequencing revealed enrichment of exhausted CD8⁺ T cells, regulatory T cells, M2-like tumor-associated macrophages, and tolerogenic LAMP3⁺ dendritic cells, accompanied by depletion of cytotoxic effector populations. Trajectory analyses suggested a transition from pre-exhausted to terminally exhausted T-cell states driven by TOX and immune checkpoint signaling. Several suppressive ligand-receptor interactions, including IL1B-IL1R2 and HLA-I-LILRB1, were identified as potential targets for restoring antitumor immunity, underscoring ESCC-specific immune escape mechanisms (Zheng et al., 2020). Stromal heterogeneity has emerged as another critical determinant of ESCC progression. scRNA-seq studies identified a tumor-enriched CST1⁺ myofibroblast population associated with extracellular matrix remodeling and poor prognosis, as well as an antigen-presenting MHC II⁺ fibroblast subset with potential immunomodulatory roles (Dinh et al., 2021). Expanding these findings, large-scale profiling of over 200,000 single cells from 60 ESCC patients delineated multiple malignant epithelial programs including proliferative, stress-response, antigen-presentation, mucosal-like, and EMT-like states and confirmed a predominantly immunosuppressive microenvironment enriched for exhausted T cells and tolerogenic dendritic cells (Zhang et al., 2021). Notably, a mucosal-like transcriptional program characterized by CXCL17, AGR2, and MUC20 expression correlated with improved survival, highlighting prognostically relevant tumor cell states unique to ESCC.

Recent studies further extend single-cell insights toward therapeutic stratification and biomarker development. Deep immune profiling identified 53 immune cell clusters and demonstrated that PD-L1 blockade can partially reprogram tumor-associated macrophages toward a pro-inflammatory phenotype. Importantly, CD39 (ENTPD1) was shown to more accurately mark tumor-reactive T cells and predict anti-PD-1 response than PD-1 or CD103 alone, suggesting a refined biomarker for immunotherapy selection in ESCC (Chen et al., 2025). In parallel, characterization of the vascular niche revealed a metastatic-enriched GPR116⁺ pericyte subset transcriptionally driven by PRRX1. These pericytes secrete EGFL6, which activates integrin β 1-NF- κ B signaling in cancer cells to promote invasion and metastasis; targeting this axis suppressed metastasis and enhanced immunotherapy efficacy in preclinical models, while circulating EGFL6 emerged as a potential noninvasive diagnostic and prognostic biomarker (Pei et al., 2025). Collectively, these ESCC-focused single-cell studies move beyond descriptive atlases to reveal disease-defining cellular states, immune escape circuits, and stromal-tumor interactions. While scRNA-seq currently remains a

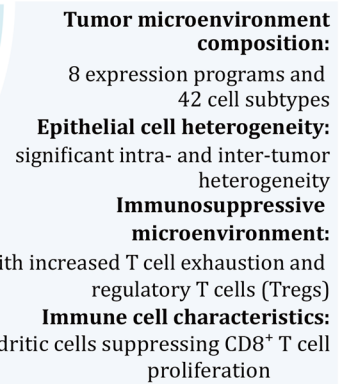


Figure 3. Overview of scRNA-seq workflow and major findings in ESCC. This schematic illustrates the general workflow of scRNA-seq, including tissue dissociation, single-cell isolation, library construction, sequencing, and data analysis. It also summarizes the key discoveries from scRNA-seq studies in ESCC, highlighting pronounced tumor cell heterogeneity, metabolic reprogramming (e.g., glycolysis and OXPHOS), and a predominantly immunosuppressive tumor microenvironment. Major findings include T-cell exhaustion with upregulated immune checkpoints, macrophage polarization from M1 to M2, distinct B cell and dendritic cell states, tumor-specific CST1⁺ myofibroblasts associated with poor prognosis, antigen-presenting fibroblasts, and complex cell-cell communication networks shaping ESCC progression.

research-intensive platform, the recurrent identification of prognostic tumor programs, immune exhaustion markers, and targetable stromal signaling pathways provides a rational foundation for translating single-cell discoveries into clinically actionable biomarkers and therapeutic strategies for ESCC.

Proteomic alterations reflect ESCC molecular complexity

Proteomics provides a functional layer of molecular information that complements genomic and transcriptomic findings, offering direct insight into dysregulated pathways and tumor behavior. With advances in mass spectrometry (MS), including quantitative strategies such as stable isotope labeling with amino acids in cell culture (SILAC), isotope-coded affinity tag (ICAT), isobaric tags for relative and absolute quantitation (iTRAQ), tandem mass tags (TMT), as well as label-free methods, researchers are now able to characterize ESCC proteomes with increasing depth and accuracy. These technologies, together with

emerging proximity-based protein assays, have facilitated the detection of low-abundance proteins and enabled biomarker discovery from limited clinical material.

Early proteomic studies using two-dimensional difference gel electrophoresis (2D-DIGE) identified proteins involved in structural integrity and cell adhesion, such as a 195-kDa perimembrane protein downregulated in ESCC (Nishimori et al., 2006). TGM3 was subsequently recognized as a prognostic biomarker, with higher expression associated with better survival (Uemura et al., 2009), while elevated PTMA correlated with tumor progression (Zhu et al., 2019b).

Quantitative proteomic approaches have further expanded the ESCC protein landscape. iTRAQ-based analyses identified candidate biomarkers including PSAP, PDIA4, and PLEC1 from paired tumor-normal samples (Pawar et al., 2011), and revealed additional underreported proteins such as proline 4-hydroxylase subunits (Singh et al., 2021). TMT-based profiling uncovered overexpression of ILK, whose inhibition suppressed ESCC proliferation, migration, and invasion, suggesting therapeutic

relevance (Ning et al., 2020). Other studies highlighted dysregulated pathways: increased RNA transcription and metabolic activity linked FBL expression to prognosis (Jin et al., 2021), while large-cohort TMT datasets identified thousands of differentially expressed proteins enriched in cell cycle control, DNA repair, immune modulation, EMT, and metabolic reprogramming (Liu et al., 2021a). Proteomic profiling in advanced ESCC further emphasized aberrant cell cycle and spliceosome activity, alongside downregulation of ECM-receptor and focal adhesion pathways (Li et al., 2021c), collectively underscoring profound remodeling of structural and signaling networks during tumor progression. Together, these studies demonstrate that ESCC proteomics not only reveals key tumor-associated pathways but also identifies clinically relevant biomarkers and potential therapeutic targets, strengthening its translational significance.

Posttranslational modifications influence ESCC biology

Utilizing omics technologies enables the comprehensive detection of a diverse array of posttranslational modifications (PTMs), including phosphorylation, acetylation, succinylation, ubiquitination, glycosylation, sulfation, lipidation, hydroxylation, SUMOylation, nitrosylation, and ADP-ribosylation (Fig. 4). These modifications play pivotal roles in regulating protein function, activating or inhibiting signal transduction pathways, modulating dynamic processes, and controlling gene expression and protein degradation. Common techniques for detecting these modifications include LC-MS/MS combined with specific enrichment methods.

Phosphoproteomics reveals disrupted signaling pathways

Phosphorylation is the most extensively characterized PTM in ESCC. Large-scale phosphoproteomic analyses have uncovered extensive remodeling of kinase-regulated pathways. A label-free phosphoproteomic study quantified over 67,000 phosphorylation sites and identified thousands of dysregulated phosphosites in ESCC tissues, with enrichment in cell cycle control, EMT, estrogen response pathways, and hyperphosphorylation of spliceosome-associated proteins (Liu et al., 2021a). Another study profiling advanced-stage ESCC identified 1,691 DEPSs, with upregulated phospho-proteins clustering in cell cycle and spliceosome pathways, whereas phosphorylation levels within ECM-receptor and focal adhesion pathways were reduced (Li et al., 2021c). Together, these findings underscore aberrant phosphorylation signaling as a major driver of ESCC progression.

Glycoproteomics identifies aberrant glycosylation patterns

Aberrant glycosylation is increasingly recognized as a hallmark of ESCC. Quantitative N-glycoproteomic profiling

identified dysregulated glycosylated proteins, including LAMP2, PLOD2, and fucosylated forms of ITGB1 and CD276, which may serve as biomarkers of tumor progression (Gao et al., 2022). Serum IgG glycopatterns further distinguished ESCC, precancerous lesions, and healthy controls, with a composite glycan score showing strong predictive ability and implicating fucosylation and mannose suppression as potential therapeutic targets (Pan et al., 2023). Additional studies identified recurrently altered N-glycoproteins such as procathepsin D and clusterin (Liu et al., 2022b), while salivary glycomic profiling revealed diagnostic N-glycan motifs recognized by DSA lectin (Shu et al., 2021). Database-driven analyses also highlighted GLUT1-associated sialyl-Tn glycoforms as biomarkers for ESCC subtype stratification (Cotton et al., 2021). These results collectively indicate that glycan remodeling plays a functional and clinically relevant role in ESCC.

Acetylproteomics maps acetylation changes

Lysine acetylation, a key regulator of chromatin structure and transcription, is also altered in ESCC. SILAC-based acetylproteomic profiling in ESCC cell models revealed multiple histone PTMs including acetylation, methylation, and a novel butyrylation event, with H4K79me2 identified as a distinctive modification associated with tumor aggressiveness (Zhang et al., 2015a). These findings highlight acetylation-driven chromatin changes as contributors to ESCC biology.

Succinylproteomics uncovers novel regulatory mechanisms

Beyond phosphorylation, glycosylation, and acetylation, emerging evidence indicates that additional lysine-based PTMs, particularly succinylation, also contribute to ESCC biology. Succinylation markedly alters lysine residue charge and structure, thereby influencing protein function and cellular metabolism. An integrated multi-omic comparison of immortalized esophageal epithelial cells (SHEE) and their malignantly transformed counterparts (SHEEC) revealed a global reduction in lysine succinylation in ESCC cells (Guo et al., 2021). This widespread loss of succinylation was associated with metabolic reprogramming and enhanced migratory capacity, suggesting that succinylation may act as an unrecognized regulatory node in ESCC pathogenesis and could represent a novel therapeutic vulnerability.

Although several PTMs have now been profiled, many biologically important modifications including lipidation, S-nitrosylation, sulfation, hydroxylation, and ubiquitination remain largely unexplored in ESCC. These PTMs regulate essential processes such as membrane targeting, nitric oxide signaling, protein-protein interactions, collagen stability, and proteasomal degradation. Systematic investigation of these unexplored PTMs will be critical for establishing a more complete ESCC PTM atlas and may reveal additional biomarkers or therapeutic targets.

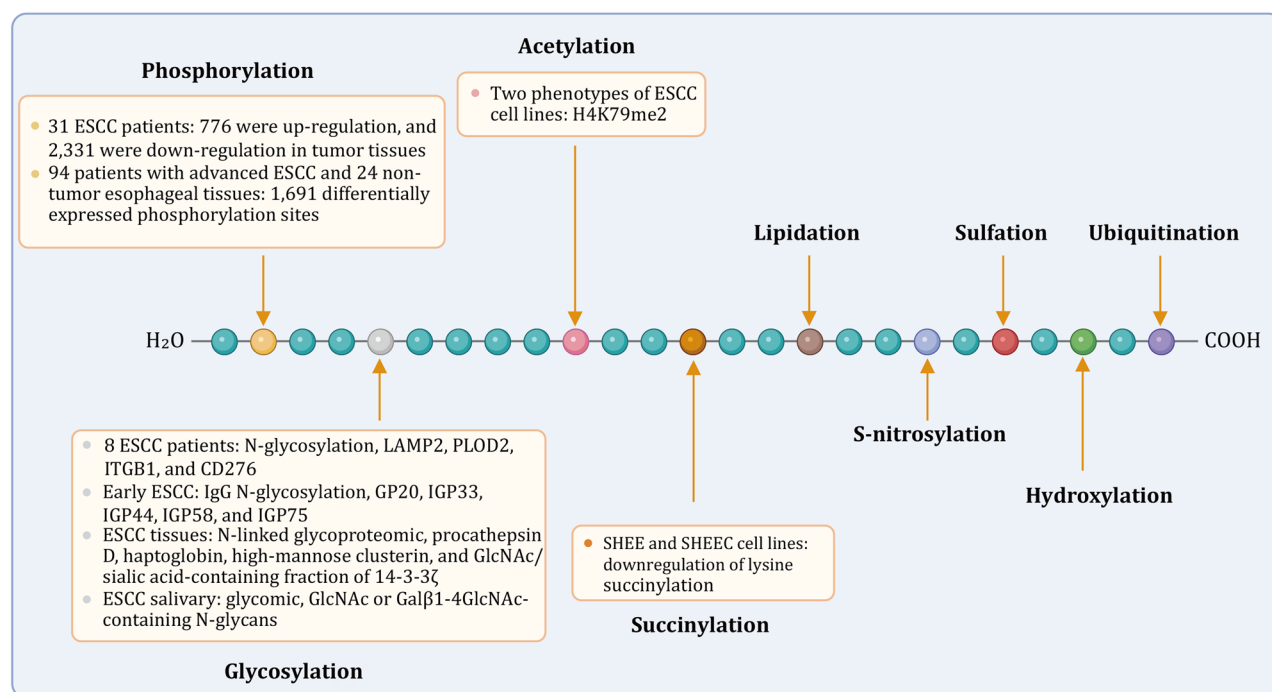


Figure 4. Representative research progress on PTMs in ESCC. This figure summarizes the major types of PTMs studied in ESCC, including phosphorylation, acetylation, methylation, ubiquitination, glycosylation, sulfation, lipidation, and hydroxylation. To date, PTM research in ESCC has mainly focused on phosphorylation, glycosylation, acetylation, and succinylation.

Metabolic reprogramming characterizes ESCC

Metabolic reprogramming is a hallmark of ESCC, reflecting the extensive biochemical shifts that sustain malignant transformation, progression, and metastasis. Enabled by analytical platforms such as nuclear magnetic resonance (NMR), MS, ultra-performance liquid chromatography (UPLC), and liquid chromatography-tandem mass spectrometry (LC-MS/MS), metabolomics has uncovered widespread alterations in amino acid, phospholipid, nucleotide, and lipid metabolism in ESCC, offering new opportunities for biomarker discovery and mechanistic insight.

Early plasma- and urine-based metabolomic studies revealed broad disruptions in amino acid pools, phospholipid metabolites, and small-molecule intermediates. Reduced circulating levels of multiple amino acids (Asp, Glu, Gly, His, Thr, Tau, Ala, Met, Ile, Leu, Phe) together with elevated Cys were observed in ESCC patients (Ma et al., 2014), while UPLC-TOF-MS profiling identified increased phospholipid species including phosphatidylserine, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, and sphinganine 1-phosphate—in ESCC plasma (Liu et al., 2013). Urine metabolomics further identified pyroglutamic acid, indole, and p-xanthine as candidate diagnostic metabolites (Xu et al., 2013). Studies comparing metastatic and nonmetastatic ESCC patients showed metabolic shifts involving glycolysis, glutaminolysis, lipid turnover, branched-chain amino acid catabolism, and redox pathways, suggesting metabolic

signatures associated with disease aggressiveness (Jin et al., 2014).

Spatial metabolomics has provided additional insight into intratumoral heterogeneity. AFADESI-MSI mapping of tissues from 256 ESCC patients highlighted regional dysregulation in proline biosynthesis, glutamine and uridine metabolism, histidine pathways, fatty acid synthesis, and polyamine metabolism, pointing to PYCR2 and UPase1 as functionally relevant metabolic drivers (Sun et al., 2019). Targeted tryptophan metabolomics also implicated tryptophan-kynurenine axis alterations in both ESCC development and metastasis (Cheng et al., 2017). Lifestyle factors may further shape metabolic risk: smoking was linked to a 3.11-fold increase in ESCC risk, potentially mediated by changes in glutamine, histidine, and cholic acid metabolism (Wei et al., 2021).

Integrated metabolomics-transcriptomics approaches have strengthened biomarker discovery. Dysregulation of glycerophosphatidylcholine metabolism in ESCC tissues led to the identification of PTDSS1 and LPCAT1 as candidate diagnostic genes (Yang et al., 2022), while another tissue-based UPLC/MS study revealed consistent upregulation of amino acid transporters SLC7A5, SLC1A5, and SLC16A10, implicating nutrient transport pathways as potential therapeutic targets (Chen et al., 2021a). Exosome-derived metabolomics further broadened diagnostic potential: profiling of 196 plasma exosome metabolites identified 3'-UMP, palmitoleic acid, palmital, and

isobutyl decanoate as predictors of recurrence (Zhu et al., 2021a). A large UPLC-MS/MS study of 450 ESCC patients revealed retinol and linoleic acid metabolism as actionable therapeutic pathways (Wang et al., 2022c). Consistently, plasma GC-MS profiling from patients with ESCC, esophageal squamous dysplasia, and controls demonstrated progressive metabolic changes, including α -tocopherol, cysteine, and aminomalonic acid alterations across disease stages (Yu et al., 2022). Complementary ^1H -NMR profiling identified creatine and glycine as promising noninvasive liquid biopsy markers (Ouyang et al., 2023).

Together, these studies (summarized in Table 4) demonstrate that ESCC is characterized by profound metabolic rewiring across amino acid metabolism, lipid remodeling, nucleotide pathways, and TME interactions. Metabolomics thus offers both mechanistic insights and a growing repertoire of diagnostic and prognostic biomarkers.

Multi-omics integration elucidates ESCC molecular alterations

The integration of multi-omics data has greatly advanced understanding of ESCC by linking genomic alterations with downstream transcriptional, proteomic, phosphoproteomic, and metabolic changes. Rather than interpreting each omics layer independently, recent studies have demonstrated that combined analyses can uncover convergent pathways, identify actionable vulnerabilities, and refine molecular subtypes with clinical relevance.

One representative study simultaneously profiled the transcriptome, proteome, phosphoproteome, and metabolome in 24 paired ESCC and adjacent tissues, revealing coordinated dysregulation of RNA processing, cell cycle control, and metabolic pathways, and nominating several potential therapeutic targets (Jin et al., 2021). A larger proteogenomic investigation comparing proteomes of 124 paired tissues with phosphoproteomes of 31 paired tissues and previously reported genomic datasets identified alterations in 35 significantly mutated genes. Among them, TP53 protein levels were elevated, whereas ERBB2 and ZNF750 were reduced, supporting their functional roles in ESCC progression. Integrated pathway-level analyses further highlighted aberrant RTK-PI3K, WNT, and Notch signaling, alongside frequent mutations affecting pericyte-associated components (Liu et al., 2021a). At the proteomic and phosphoproteomic level, profiling of 94 primary tumors and 24 nontumor tissues identified CLK1 as a therapeutically relevant node associated with patient prognosis (Li et al., 2021c). Another multi-omics analysis of transcriptome, proteome, and phosphoproteome data from 60 treatment-naïve paired ESCC samples identified TIMMDC1 as a prognostic indicator and CSNK2A1 as a promising drug target in ESCC biology (Zhao et al., 2024).

Large-scale integrative studies have further mapped ESCC carcinogenesis at the systems level. Analysis of 786 tumor samples from 154 patients using genomic, proteomic, and phosphoproteomic profiling revealed six developmental trajectories representing progressive alterations in major cancer pathways; phosphorylation of PGK1 at S203 emerged as a potential therapeutic target (Li et al., 2023b). Similarly, integration of epigenomic, transcriptomic, and proteomic datasets from 155 ESCC cases identified four molecular subtypes, cell cycle activation, NRF2 pathway activation, immunosuppression (IS), and immunomodulation (IM). The IS and IM subtypes exhibited high immune infiltration, and patients classified as IM showed superior responses to immune checkpoint blockade therapies in clinical trials. A 28-feature classifier developed from these datasets predicted IM subtype and anti-PD-1 responsiveness with a sensitivity of 85.7% and specificity of 90% (Liu et al., 2023b).

Collectively, these studies illustrate how multi-omics integration uncovers functionally relevant pathways, identifies biomarkers and therapeutic targets, and refines ESCC molecular taxonomy with translational implications. Representative multi-omics investigations are summarized in Table 5.

Multi-omics reveals translational potential in ESCC

A series of published studies on the omics landscape of ESCC have provided valuable insights for understanding the pathogenesis, molecular subtyping, therapeutic targets, prognostic biomarkers, diagnostic indicators, tumor heterogeneity, TME, and precision treatment of ESCC (Fig. 5). Representative studies have been chosen to exemplify each application.

Multi-omics uncovers key oncogenic mechanisms

Beyond descriptive cataloging, multi-omics analyses have elucidated mechanistic insights into ESCC initiation and progression. For instance, studies reported that chromosome 3q gains occur during the shift from non-neoplastic tissue to intraepithelial neoplasia, while TP53 mutations augment DNA replication during this transition (Li et al., 2023b). Alterations such as AKAP9 and MCAF1 mutations promote glycolysis and WNT signaling, respectively, at later stages. Regulatory networks have also been uncovered. Wang et al. identified miR-340-5p as a suppressor of PIK3C3, showing that its inhibition promotes proliferation while PIK3C3 re-expression reverses this effect (Wang et al., 2020b). Proteogenomic analysis revealed ribosomal protein FBL as an oncogenic driver that enhances PI3K/AKT activation and accelerates cell cycle progression (Jin et al., 2021). Importantly, Omics approaches can identify dysregulated signaling pathways in ESCC such as PI3K/

Table 4. Representation of metabolomics studies in ESCC.

Cancer type	Platform	Number of samples	Sample sources	Up metabolite	Down metabolite	References
ESCC	LC-MS/MS	n = 92	Serum	Phenylalanine, 4-hydroxyphenyllactic acid, 3,4-dihydroxyphenylalanine, 3,4-dihydroxyphenylacetic acid and tyrosine Cystine	NA	(Cheng et al., 2021)
ESCC	HPLC	n = 60	Plasma		Aspartate, glutamate, glycine, histidine, threonine, taurine, alanine, methionine, isoleucine, leucine, phenylalanine Desmosine/Isodesmosine, 5- β -cyprinol sulfate	(Ma et al., 2014) (Liu et al., 2013)
ESCC	UPLC-ESI-TOFMS	n = 53	Plasma	Phosphatidylinositol, lithocholyltaurine, phosphatidic acid, L-Uroilinogen, 9'-carboxy-gammatocotrienol, phosphatidyl choline, phosphatidyl ethanolamine, sphinganine 1-phosphate, phosphatidylserine, lysoPC, ganglioside GM2, ganglioside GM2, 12-oxo-20-dihydroxy-leukotriene B4		
ESCC	RRLC-MS/MS SRM	n = 44	Plasma	L-Carnitine, lactic acid, uric acid, citric acid, linolenic acid, linoleic acid, oleic acid, arachidonic acid	Octanoylcarnitine, nonanoylcarnitine, decanoylcarnitine, undecanoylcarnitine, lysoPC (14:0), lysoPC (16:1), lysoPC (16:0), lysoPC (18:0), lysoPC(20:3), cholic acid Glucose, alanine, lactic acid, glutamine, citric acid, fumaric acid, 1,5-Anhydroglucitol, valine, 2-Ketoisovaleric acid, 2-Ketoisocaproic acid, 3-Methyl-2-oxovaleric acid, tryptophan, indolelactic acid, iminodiacetic acid, phosphoethanolamine, γ -Aminobutyric acid, glycolic acid, cysteine, methylcysteine, α -Tocopherol, γ -Tocopherol, threonine, uric acid, erythritol, inositol, myo-Inositol 1-phosphate, cholesterol, hydroxylamine	(Xu et al., 2013) (jin et al., 2014)
ESCC	GC/MS	n = 80	Serum	2-Hydroxybutyric acid, hypotaurine, aspartic acid, β -alanine, 3-Hydroxybutyric acid, myristic acid, palmitic acid, oleic acid, linoleic acid, palmitoleic acid, 1-Monooleoylglycerol, ribose, maltose, lactose, creatinine		
ESCC	AFADESI-MSI	n = 256	Tissue	Arginine and proline metabolism; FA biosynthesis; alanine, aspartate; glutamate metabolism; pyrimidine metabolism; histidine metabolism 5-hydroxytryptamine	NA	(Sun et al., 2019)
ESCC	UPLC-MS/MS	n = 38	Serum		Tryptophan	(Cheng et al., 2017)
ESCC	UHPLC-QTOF/MS	n = 464	Serum	Glutamine, histidine, cholic acid	NA	(Wei et al., 2021)
ESCC	UHPLC-QTOF/MS	n = 60	Tissues	Glycerophospholipid metabolism		(Yang et al., 2022)
ESCC	UPLC/MS	n = 141	Tissues	Ascorbic acid, formylkynurenine, adeny/succinic acid, kynurenine, 5-hydroxy-1-tryptophan	Dihydroxyacetone phosphate, ricinoleic acid, 2-hydroxyadipic acid, galactose, and 3-hexaprenyl-4-hydroxybenzoic acid. Palmitoleic acid	(Chen et al., 2021a)
ESCC	LC-ESI-MS/MS	n = 91	Plasma	3'-UMP, palmitaldehyde		(Zhu et al., 2021a)
ESCC	GC/MS	n = 373	Plasma	Aminomalonic acid	Alpha-tocopherol, cysteine	(Yu et al., 2022)
ESCC	LC-MS	n = 62	Urine	Pyroglutamic acid, indoxyl, urocanic acid, L-Carnitine, L-Fucose, uric acid, acetyl/carnitine, deoxycytidine, phenylacetylglutamine, cAMP, cGMP	Paraxanthine, heptanoylcarnitine, octenoylcarnitine, nonenoylcarnitine, nonanoylcarnitine, decanoylcarnitine, undecenoylcarnitine, undecanoylcarnitine	(Xu et al., 2016)

(Continued)

Table 4. Continued.

Caner type	Platform	Number of samples	Sample sources	Up metabolite	Down metabolite	References
ESCC	¹ H-NMR	n = 89	Tissues	NAA, glycine, glutamate, valine, leucine/isoleucine, L-tyrosine, phenylacetylglycine, methionine, phenylalanine, GABA, phenylacetylglutamine, glutamate γ -H, taurine, glycoprotein, acetone, malonate, acetoacetic acid, acetate, trimethylamine, formate, uracil, NAC1, adenine in ATP/ADP and NAD/NADH, NAC2, acetyl hydrazine, hippurate	Creatine, glycine, glutamine, 4-HPPA, phenylacetylglycine, creatinine, L-aspartate, myo-inositol, Cho, choline, glucose, ethanol, α -ketoglutaric acid oxime, AMP, NAD	(Ye et al., 2021b)
ESCC	GC/MS LC/MS	n = 26	Serum	Glutaric acid, cystine, betaine, glucuronic acid	Xylose, pyruvic acid	(Nishiiumi et al., 2019)
ESCC	GC/MS, LC/MS	n = 26	Serum		Arabitol, betaine, glycine, L-aspartate, L-serine, 3-aminoglutaric acid, L-arginine, uracil, 2-hydroxyglutaric acid	(Fujiigaki et al., 2018)
ESCC	UPLC-MS/MS	n = 50	Urine	Nicotinuric acid, fucose, urocanic acid, phenylacetylglutamine, cAMP, cGMP	Glucuronide	(Xu et al., 2019)
ESCC	GC/ UPLC-TQ-MS/MS	n = 67	Serum	Tryptophan (Trp), L-carnitine, acetyl-carnitine	Citrulline, lysine	(Zhao et al., 2022c)
ESCC	GC-TOF/MS	n = 34	Sera	Glutamine	Glutamic acid, alanine, glycine, serine, ornithine, ribose, glyceric acid, docosahexaenoic acid, linoleic acid	(Liu et al., 2022a)
ESCC	LC-MS/MS	n = 40	Serum	Phosphatidylcholines	NA	(Mir et al., 2015)
ESCC	GC-TOF/MS	n = 46	Plasma	Isocitric acid, linoleic acid, citric acid	L-histidine, 3'4 dihydroxyhydrocinnamic acid	(Zhang et al., 2020b)
ESCC	GC-MS	n = 74	Serum	L-glutamine	Propanoic acid, linoleic acid, glycerol-3-phosphate	(Zhang et al., 2020a)
ESCC	CE-MS and LC-MS	n = 28	Tissues	Leucine, isoleucine, valine	NA	(Zang et al., 2021a)
ESCC	UHPLC-QTOF/MS	n = 653	Serum	Hypoxanthine, inosine, carnitine (14:1), glycochenodeoxycholate, PC (P-18:0/18:3)	Dopamine, L-histidine, 5-hydroxyindoleacetate, L-tryptophan, 2'-O-methylcytidine, PC (14:0/0:0), PC (O-16:1/0:0), PE (18:0/0:0), PC (16:1/0:0), PC (18:2/0:0)	(Li et al., 2021b)
ESCC	LC-MS	n = 88	Plasma	Arachidonic acid, piperidine, carnitine, 7Z, 10Z, 13Z, 16Z, 19Z-docosapentaenoic acid, sebacic acid, acetoacetic acid	Indoxyl sulfate, PC (14:0/0:0), deoxycholic acid, trimethylamine N-oxide, pipecolic acid, hydrocinnamic acid	(Chen et al., 2021b)
ESCC	¹ H-NMR	n = 65	Serum, urine	Leucine, isoleucine, arginine, valine, lysine, glutamate, glutamine, asparagine, choline, phosphocholine and glycine	Lactate, alanine, pyruvate, citrate, creatine, taurine, myo-inositol and glucose	(Liu et al., 2019)
ESCC	MALDI-MSI	NA	Tissue	Cholines, amino acids, polyamines, carnitines, nucleosides, organic acids, phosphatidylcholines (PCs), fatty acids (FAs), phosphatidylethanolamines (PEs), phosphatidic acids (PAs), phosphatidylserines (PSs), phosphatidylglycerols (PGs) and phosphatidylinositols (PIs)	NA	(Zang et al., 2021b)

NA, not provided by the author

Table 5. Representation of multi-omics study in esophageal squamous cell carcinoma.

Cancer type	Omic analysis	Platform	Sample sources	No. of samples	Key genes	References
ESCC	Genome	WES, TCR repertoire sequencing	Tissue, blood	WES (n=39), TCR repertoire sequencing (n=39)	ERBB, BRCA1/2, CD274	(Yan et al., 2019)
ESCC	Genome, Epigenome, Transcriptome	WGS, WGBS, RNA-seq, iTRAQ	Tissue	WGBS (n=20), WGS (n=6), RNA-seq (n=20), and iTRAQ (n=20)	H3K9me3, H3K27me3, EZH2/SUZ12, lncRNA ESCCAL-1	(Cao et al., 2020)
ESCC	Genome	DNA methylation	Tissue	DNA methylation (n=91)	cg23378365, cg06090867 and cg03244277 in the promoters of CYFIP2, UBXN10, AREG, respectively, and cg02370667 in the gene-body of NECAB2	(Xi et al., 2022a)
ESCC	Genome, Transcriptome	WGS, RNA-Seq	Tissue, peripheral blood samples	WGS (n=94), RNA-Seq (n=94)	LRP1B, TTC28	(Chang et al., 2017)
ESCC	Genome, Transcriptome, epigenome	WES, RNA-Seq, DNA methylation	Tissue	WES (n=88 pairs), RNA-Seq (n=57), DNA methylation (n=67)	NOTCH family genes, RTK/PI3K pathway genes, and NFE2L2 pathway genes	(Takemoto et al., 2021)
ESCC	Genome, Transcriptome	WGS, RNA-Seq	Tissue	WGS (n=528), RNA-Seq (n=133)	PTHLH	(Cui et al., 2022)
ESCC	Genome, Transcriptome	WES, RNA-Seq	Tissue, blood	WES (n=50), RNA-Seq (n=125)	TP53, PIK3CA	(Liu et al., 2020b)
ESCC	Transcriptome, Proteome, Phosphoproteome, Metabolome	RNA-Seq, iTRAQ, GC-MS	Tissue	RNA-Seq (n=24), iTRAQ (n=24), GC-MS (n=24)	FBL	(Jin et al., 2021)
ESCC	Proteome, Phosphoproteome	iTRAQ, TMT	Tissue	iTRAQ (n=124), TMT (n=31)	ELOA, SCAF4	(Liu et al., 2021a)
ESCC	Proteome, Phosphoproteome	iTRAQ	Tissue	iTRAQ (n=94/24)	CLK1	(Li et al., 2021c)
ESCC	Transcriptome, Proteome, Phosphoproteome	RNA-seq, TMT	Tissue	RNA-seq (n=60), TMT (n=6)	TIMMDC1, CSNK2A1	(Zhao et al., 2024)
ESCC	Genome, Proteome, Phosphoproteome	WES, label-free	Tissue	WES (n=102), label-free (n=786), label-free (n=145)	TP53, AKAP9, MCAF1, PGK1	(Li et al., 2023b)
ESCC	Genome, Epigenome, Transcriptome, Proteome,	WGS, WGBS, RNA-seq, scRNA-seq, proteomics	Tissue	WGS (n=155), WGBS (n=155), RNA-seq (n=155), scRNA-seq (n=155), proteomics (n=73)	CCND1, CDKN2A/B, NFE2L2, KEAP1, CUL3, SOX2, CD56, ERBB2	(Liu et al., 2023b)

AKT signaling, Src/FAK signaling, RAS/RAF signaling. Together, these studies demonstrate how multi-omics can resolve stage-specific events and reveal functional convergence on metabolic remodeling, DNA replication stress, and kinase signaling.

Molecular subtypes of ESCC emerge from integrative clustering

Unsupervised clustering across genomic, transcriptomic, and epigenomic layers has revealed the presence of distinct ESCC molecular subtypes. In Japanese cohorts, two mutational signatures—CpG transitions and APOBEC-mediated substitutions—were linked to environmental exposures (alcohol, smoking) and genetic variants (ALDH2 and CYP2A6) (Sawada et al., 2016). TCGA further classified esophageal cancers into ESCC1, ESCC2, and ESCC3, with

clear geographic and molecular distinctions (Cancer Genome Atlas Research Network et al., 2017). Across 1,930 ESCC genomes, an ESCC-meta-set identified 11 mutational signatures and three NMF clusters. Cluster 1, enriched for APOBEC signatures and ZNF750 mutations, exhibited the poorest prognosis and greater metastatic propensity, whereas cluster 3 was characterized by mismatch repair deficiency (Cui et al., 2020). Another study defined three conserved subtypes based on mutational signature activity, with subtype-specific enrichment of CDC27 amplification, ZNF750 mutations, or TP53 dominance (Guo et al., 2018).

Transcriptomic subtyping has similarly identified clinically meaningful groups. GSEA-based classification of 141 tumors defined three subtypes with dominant feature, WNT pathway activation, glycogen-metabolic suppression, and attenuated neutrophil degranulation (Wang

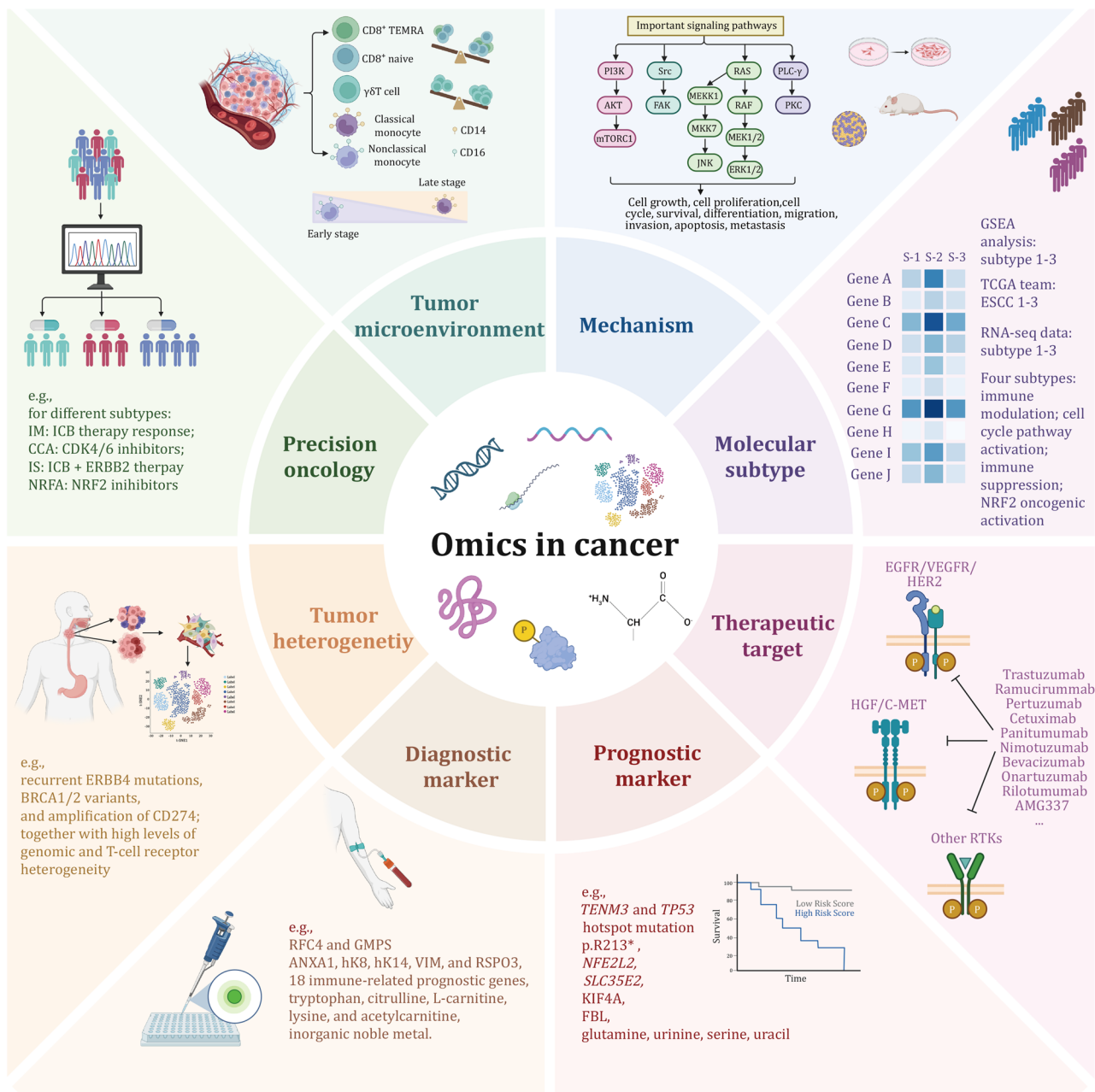


Figure 5. Multi-omics reveals translational potential in ESCC. This schematic summarizes how integrative multi-omics analyses (including genomics, transcriptomics, proteomics, metabolomics, and epigenomics) uncover the translational significance of ESCC. Multi-omics approaches elucidate tumor heterogeneity, molecular subtypes, and tumor microenvironmental features; dissect key oncogenic signaling mechanisms; and enable the identification of diagnostic and prognostic biomarkers. In addition, these data support precision oncology by guiding patient stratification and informing the discovery of actionable therapeutic targets, thereby bridging molecular insights with clinical applications in ESCC.

et al., 2022b). Additional RNA-seq analyses described metabolic, inflammatory/metastatic, and cell-proliferative subtypes, distinguished by alterations in EMT pathways, integrin/ECM signaling, and MYC-driven cell cycle programs (Liu et al., 2020b). Recent large-scale integrative work further grouped ESCC into four multi-omics subtypes, cell cycle activation, NRF2 oncogenic activation, IS, and IM. Notably, IM tumors displayed high immune infiltration and superior responses to checkpoint blockade in

clinical trials, enabling the development of a 28-feature classifier that predicts anti-PD-1 responsiveness with high sensitivity and specificity (Liu et al., 2023b). Additionally, Japanese ESCC cases were clustered into two mutational signatures: an APOBEC-associated signature and an age-related signature (Takemoto et al., 2021). Collectively, these subtyping systems highlight the molecular heterogeneity of ESCC and offer promising frameworks for patient stratification.

Table 6. Representation of diagnostic marker, therapeutic target, and prognostic marker in ESCC.

Omics	Function	Key molecular
Genomics	Diagnostic marker	12-marker diagnostic panel, RFC4, GMP5
	Therapeutic target	EGFR, BRCA2, BRCA1, ROS1, ALK, ERBB2, MET, BRAF, NTRK1, FGFR1, KRAS, RET, CD274, VEGFA, SLC7A8, ZNF750, XPO1, PIK3CA, FAT1, ADGB, DOPEY1, HECW1, LAMA1, THSD7A, USP9Y, PRKAG2, PLCH2, NP1A5, MIB2, ERBB4, FGFR2, ATM, TP53
	Prognostic marker	EP300, TET2, FAM135B, SLC39A, 4 promoter/gene-body CpG sites, APOBEC-associated signatures, NFE2L2, TP53 hotspot mutation p. R213*, SLC35E2, TP53
Epigenetics	Diagnostic marker	PAX9, SIM2, THSD4, IL22RA2, TNFSF13B, SERPINA4, TAC3, 12 promoter/gene body DNA methylation CpG sites, EPB41L3, GPX3, COL14A1, IL-6, MMP3, MMP9, SPP1
	Therapeutic target	HDAC, HMT, HDM, HAT, LSD1
	Prognostic marker	ABCD1, SLC5A10, SPIN3, ZNF69, ZNF608, YTHDF3, RBM15, KIAA1429, ALKBH5
Transcriptomics	Diagnostic marker	TP63, KRT5/15, BNC1, DSC3, DSG3, HERES, Linc00460, five plasma exosome lncRNAs (NR_039819, NR_036133, NR_003353, ENST00000442416.1, and ENST00000416100.1), miR-1246, serum miRNA discriminant model (miR-8073, miR-6820-5p, miR-6794-5p, miR-3196, miR-744-5p, and miR-6799-5p), miR-16-5p, miR-451a, miR-574-5p, miR-196a-5p, miR-1-3p, 4-miRNA signature, 7 serum miRNAs (miR-10a, miR-22, miR-100, miR-148b, miR-223, miR-133a, and miR-127-3p), Five miRNAs (hsa-mir-103-1, hsa-mir-18a, hsa-mir-324, hsa-mir-369, and hsa-mir-320b-2), ESCCAL_1, HOTAIR, miR-424, hsa-miR-103/107, circIMMP2L, circGSK3β, EGFL6
	Therapeutic target	PTGES, GRB7, ODC1, POSTN, ASPA, CCND1, SF3B4, miR-130b, NSUN2, PTTG1, WDR66, Rab25, ZNF750, AMPK, Lnc-KIAA1244-2, m7G-lncRNAs, lncRNA-TTN-AS1, miR-133b, FSCN1, MMP12, Lnc-KIAA1244-2, HERES, miR-424, miR-145-5p, miR-152, miR-193b-3p, and miR-376a-3p, miR-31, IL1B-IL1R2, HLA-I-LILRB1
	Prognostic marker	ANO1, MMP3, PLEK2, ZNF750, BRAP, CCND1, NF1, ASXL1, HSPA4, TGOLN2, BAIAP2, EZH2, CHAF1A, SUPT7L, COL1A1, COL3A1, CLIC3, CLIC4, MMP1, Rab25, NEK6, FBL, ZNF750, Linc00460, COL1A1, COL3A1, eleven-gene combination, BRAP, LINC00551, miR-30e, miR-124, NF1, ASXL1, HSPA4, TGOLN2, BAIAP2, EZH2, CHAF1A, SUPT7L, eight-lncRNA signature (including AP000487, AC011997, LINC01592, LINC01497, LINC01711, FENDDR, AC087045, AC137770), seven-lncRNA signature, six-lncRNA signature (AP000696.2, LINC01711, RP11-70C1.3, AP000487.5, AC011997.1, and RP11-225N10.1), m7G-lncRNAs, lncRNAs (FIRLs), three-lncRNA signature (ENST00000435885.1, XLOC_013014 and ENST00000547963.1), miRNA signature (higher levels of EVP miR-1268a and miR-4505 and lower levels of EVP miR-1972, miR-4274, miR-4701-3p, and miR-6126), four-miRNA signature, miR-1246, six-miRNA signature (has-mir-142-3p, has-mir-148b, has-mir-130b, has-mir-365a, has-mir-370, and has-mir-423), five-miRNA signature (miR-181c-5p, miR-195-5p, miR-203, miR-212-3p, and miR-28-5p), ZBTB16, AQP4, ADCYAP1R1, VIPR2, miR-182-5p, miR-455-5p, hsa_circ_0004771, KIF4A, 4-marker prognostic panel, 18 immune-related Genes, XLOC_007869, CK327190, XLOC_006476, ASLNC11164, BF894811, BQ376030, RP11-473M20.9, ESCCAL_1, HOTAIR, miR-223, miR-1269a, hsa-miR-103/107, hsa-miR-218-5p, hsa-miR-142-3p, hsa-miR-150-5p, hsa-miR-205-5p, circIMMP2L, circGSK3β, CXCL17, AGR2, MUC20, EGFL6
Proteomics	Diagnostic marker	ANXA1, hK8, hK14, VIM, RSPO3
	Therapeutic target	CSNK2A1, CLK1, PGK1, ILK
	Prognostic marker	TGM3, ILK, FBL, CLK1, TIMMDC1
PTMs	Diagnostic marker	GlcNAc or Galβ1-4GlcNAc-containing N-glycans
	Therapeutic target	Fucosylation, mannose, PGK1 S203
Metabolomics	Diagnostic marker	Phe, 4-hydroxyphenyllactic acid, 3,4-dihydroxyphe, 3,4-dihydroxyphenylacetic acid, tyrosine, PTDSS1, LPCAT1, tryptophan, citrulline, L-carnitine, lysine, acetylcarnitine
	Therapeutic target	SLC7A5/LAT1, SLC1A5/ASCT2, SLC16A10/MCT10

Therapeutic targets are identified via integrative omics

Multi-omics analyses have yielded a range of potential therapeutic targets. Loss of ZNF750 promotes angiogenesis through the DANCER/miR-4707-3p/FOXO2 axis, nominating this pathway as a therapeutic vulnerability in ZNF750-mutant tumors (Bi et al., 2020). CDCA5 activity is enhanced by promoter H3K27 acetylation, promoting proliferation, invasion, and chemoresistance (Xu et al., 2013). PLEK2 drives metastasis and chemotherapy resistance via LCN2 regulation and represents another promising target (Wang et al., 2021a). Multi-omics studies also highlight phosphoglycerate kinase PGK1 (S203 hyperphosphorylated) (Li et al., 2023b) and CLK1 (Li et al., 2021c) as actionable molecules. Additionally, mutated or aberrantly activated genes in ESCC, such as EGFR, VEGFR, HER2, c-MET, and other receptor tyrosine kinases (RTKs), have been explored

as therapeutic targets in clinical treatment (Yang et al., 2020b). These findings demonstrate how integrated omics analysis can systematically uncover therapeutic vulnerabilities that may not be apparent from single-layer datasets.

Multi-omics-based biomarkers for prognosis and diagnosis

Numerous studies have leveraged omics data to identify biomarkers predictive of prognosis, early detection, or treatment response. NFE2L2 mutations correlate with unfavorable prognosis, and recurrent noncoding driver mutations such as SLC35E2 promoter alterations further refine risk stratification (Li et al., 2021c). Overexpression of KIF4A, identified through multi-dataset analysis, also predicts poor clinical outcomes (Wang et al., 2021d). Several metabolites, including glutamine and serine, were

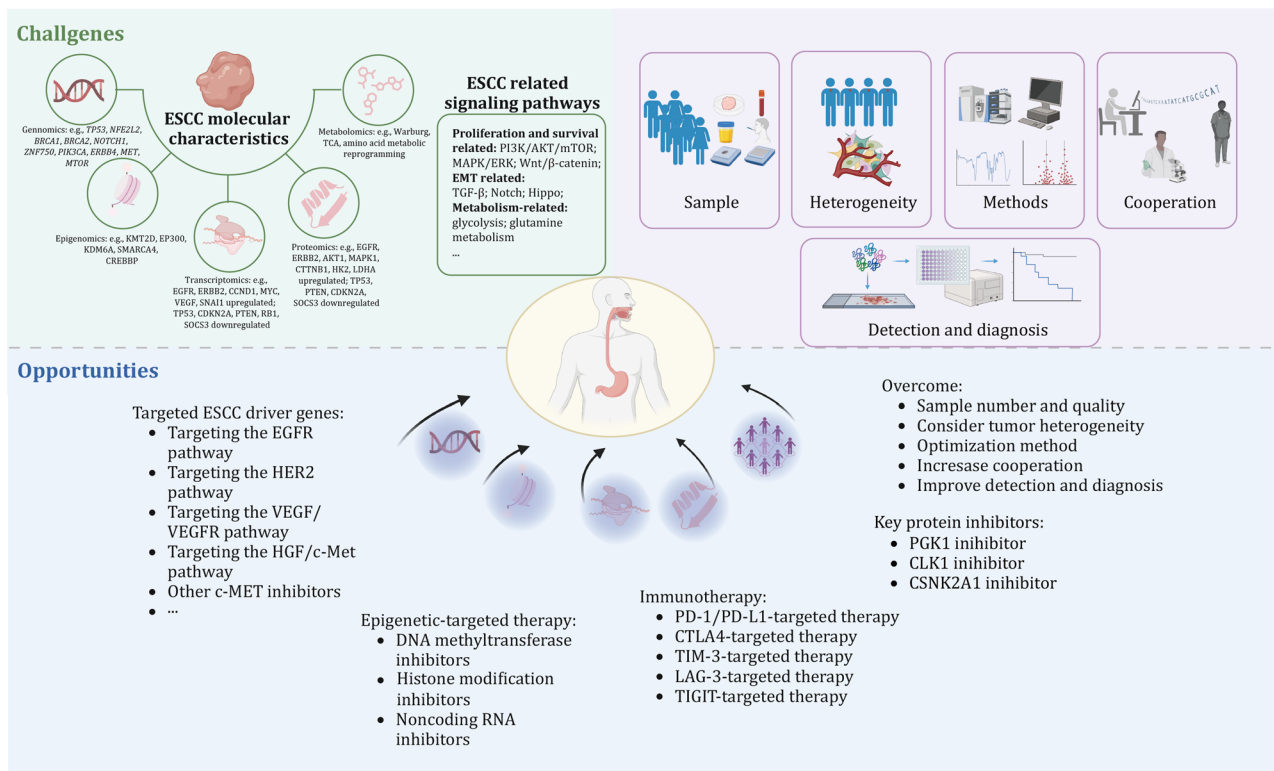


Figure 6. Opportunities and challenges of multi-omics studies in ESCC. This schematic summarizes the major challenges and opportunities associated with applying multi-omics technologies to ESCC. Multi-omics profiling reveals key molecular characteristics of ESCC across genomic, epigenomic, transcriptomic, proteomic, and metabolomic layers, and highlights dysregulated oncogenic signaling pathways involved in proliferation, metabolism, epithelial-mesenchymal transition, and immune regulation. However, clinical translation is limited by challenges including sample availability and quality, intratumoral heterogeneity, methodological variability, insufficient multicenter cooperation, and limitations in detection and diagnosis. On the opportunity side, multi-omics data enable the identification of actionable driver genes and pathways (e.g., EGFR, HER2, VEGF/VEGFR, HGF/c-MET), epigenetic targets, immune checkpoints, and key metabolic or signaling proteins, providing a foundation for targeted therapy, immunotherapy, and epigenetic-based treatment strategies.

associated with survival, revealing a metabolic layer to prognosis (Zhang et al., 2017). A 12-marker diagnostic panel and a four-marker prognostic panel have been proposed for early detection and risk assessment. Alcohol-associated mutational signatures (e.g., *TENM3*, TP53 p. R213*) serve as independent prognostic markers (Li et al., 2018).

Multi-omics has enhanced biomarker discovery beyond genomics. CNA-driven upregulation of *RFC4* and *GMPS* was linked to early ESCC detection and immune evasion (Wang et al., 2021b). Serum proteins such as ANXA1, hK8, hK14, VIM, and RSPO3 have also emerged as potential diagnostic candidates (Yang et al., 2021). RNA-seq-derived immune signatures identified 18 prognostic immune-related genes (Xiong et al., 2022). Metabolites such as tryptophan, citrulline, and L-carnitine show promise for early diagnosis (Zhao et al., 2022c). Spatial transcriptomics further identified *TAGLN2* and *CRNN* as early-warning markers experimentally shown to promote malignant transformation (Liu et al., 2023a).

Tumor heterogeneity is resolved by integrative approaches

Tumor heterogeneity encompasses both intratumor heterogeneity and intertumor heterogeneity (Lin and Lin, 2019). Intratumor heterogeneity refers to the biological variations within a single tumor, while intertumor heterogeneity pertains to the phenotypic and molecular differences between tumors in different patients (Gerashchenko et al., 2013; Tabassum and Polyak, 2015; Visvader, 2011; Wolman and Heppner, 1992). Each omics approach reveals the heterogeneity from distinct perspectives.

Multiregion WES and CGH revealed spatially distinct mutations, showing that single-region sampling fails to represent the full genomic landscape (Cao et al., 2020). Multiregion sequencing of 36 ESCC tumors (186 samples) further demonstrated heterogeneity across genomic, epigenomic, and transcriptomic levels driven by persistent chromosomal instability (Cui et al., 2023). Multiregion analysis of tumors by performing WES and TCR sequencing showed region-specific actionable targets, underscoring the clinical need for multiregion evaluation (Yan et al.,

2019). Single-cell sequencing adds another dimension by revealing immune cell and stromal heterogeneity, complementing bulk multi-omics approaches.

Multi-omics reveals microenvironmental interactions

The ESCC TME is shaped by complex interactions between cancer cells, immune populations, and stromal components. Although most immune and stromal cells generally lack recurrent genomic alterations, multi-omics studies show that they undergo profound transcriptomic and proteomic reprogramming driven by tumor-derived signals. Single-cell analyses have revealed immunosuppressive TME features, including T-cell exhaustion, regulatory T-cell accumulation, M2-like macrophage polarization, and tolerogenic dendritic cells, which jointly contribute to immune escape and therapy resistance. For example, one study revealed a reproducible immunosuppressive landscape marked by depletion of CD4⁺/CD8⁺ central memory T cells (TCM) in tumors, while functional assays showed that restoring TCM could enhance antitumor cytotoxicity. On the myeloid side, a PD-L1⁺ tumor-associated macrophage state was linked to clinical benefit, and PD-L1 blockade *ex vivo* reprogrammed TAMs toward a pro-inflammatory phenotype. Notably, CD39⁺ tumor-infiltrating T cells emerged as a TME-associated biomarker correlated with improved prognosis and better response to PD-1 blockade, highlighting its potential utility for immunotherapy stratification in ESCC (Chen et al., 2025). Integrating genomics, transcriptomics, and proteomics has enabled the mapping of ligand–receptor interactions and the identification of candidate immunotherapeutic targets.

Precision oncology is enabled by multi-omics translation

Multi-omics technologies have greatly accelerated the translation of molecular discoveries into precision oncology for ESCC by integrating genomic, transcriptomic, epigenomic, proteomic, and single-cell-level datasets. Early precision-therapy studies further demonstrate the feasibility of genomics-guided treatment selection in ESCC. Targeted agents such as gefitinib (an EGFR inhibitor) (Kang et al., 2015), dicitinib (an FGFR1 inhibitor) (Guagnano et al., 2012), and PD-L1 inhibitors (Pectasides, 2018) have shown therapeutic potential in selected patients. In addition, recent evidence indicates that CDK4/6 inhibition can enhance the efficacy of EGFR inhibitors in ESCC, suggesting promising combination-treatment strategies (Zhou et al., 2017). Recently, one study stratified ESCC into four distinct molecular subtypes with clear therapeutic implications. The CCA subtype is defined by CCND1 amplification and CDKN2A/B loss, indicating aberrant cell cycle control and suggesting sensitivity to CDK4/6 inhibitors,

such as palbociclib. The NRFA subtype is characterized by frequent alterations in NFE2L2, KEAP1, and CUL3, together with SOX2-associated activation of the NRF2 pathway, highlighting oxidative stress adaptation as a dominant feature and proposing NRF2 signaling as a potential therapeutic target. The IS subtype displays substantial immune cell infiltration but with pronounced immunosuppressive characteristics, along with relatively elevated ERBB2 (HER2) protein expression, suggesting that HER2-targeted therapy, possibly in combination with immunotherapy, may be beneficial. In contrast, the IM subtype is enriched for CD8⁺ T cells and macrophages and demonstrates the most favorable clinical response to anti-PD-1 therapy in trial settings (Liu et al., 2023b). Together, these findings illustrate how multi-omics-based stratification enables more accurate patient classification, informs rational therapeutic selection, and provides a practical framework for implementing precision treatment strategies in ESCC.

Implications of multi-omics in clinical

Among current multi-omics approaches, targeted genomics and DNA methylation profiling represent the most immediately translatable strategies for routine ESCC diagnostics, whereas proteomics, metabolomics, and single-cell approaches currently remain primarily research-oriented. In clinical practice, targeted therapies for ESCC remain limited, but multi-omics approaches have the potential to substantially improve diagnostic and therapeutic strategies by uncovering novel biomarkers, therapeutic targets, and predictors of treatment response (Table 6). Although routine immunohistochemical markers, such as P53, KI67, CK, CK5/6, P40, P63, and CK7, remain widely used, integrated analysis provides more comprehensive guidance for precision medicine, enabling better prediction of individual treatment responses and facilitating personalized therapeutic planning. NGS panels covering more than 500 genes are now widely applied to detect mutations, CNVs, and gene fusions in ESCC specimens, with platforms such as Illumina NovaSeq offering high analytical sensitivity. Actionable alterations frequently observed include ERBB2 (HER2) amplification in 3% to 8% of ESCC, which supports the use of ERBB2 inhibitors; FGFR amplifications or mutations in 8% to 10% of ESCC, suggesting eligibility for FGFR inhibitors; MET amplification (5%–8% of ESCC), predicting potential benefit from MET inhibitors; and EGFR overexpression (30%–50% of ESCC), which may predict response to EGFR inhibitors. PD-L1 and TMB testing are essential for selecting candidates for second-line immunotherapy. Postresistance testing can further reveal bypass activation of EGFR/ERBB pathways, guiding alternative treatment choices.

Several genomic alterations identified through multi-omics studies have demonstrated tangible clinical relevance. Mutations in TP53, EGFR, and ERBB2, common

in both ESCC and EAC, are now incorporated into predictive clinical decision-making. In practice, EGFR amplification supports the use of EGFR tyrosine kinase inhibitors such as gefitinib, whereas ERBB2 overexpression serves as a biomarker for trastuzumab treatment. These biomarkers can be assessed using standardized NGS assays that have become increasingly feasible for routine laboratories due to reductions in cost and turnaround time. Commercial diagnostic panels already include multiple ESCC-related genes, allowing integration of genomic testing into clinical workflows.

Future perspectives and discussion

Although multi-omics studies have greatly advanced our understanding of ESCC, revealing TP53-driven genomic instability, widespread hypomethylation, enhancer remodeling, metabolic reprogramming, and a highly immunosuppressive TME, most discoveries rely on high-cost platforms such as WGS, scRNA-seq, and spatial transcriptomics. These approaches, while mechanistically transformative, are not readily adoptable by routine service laboratories, which often lack standardized workflows, bioinformatic capacity, and access to specialized instrumentation. Consequently, a major gap persists between research-grade molecular insights and clinically deployable tools for ESCC (Fig. 6).

From the perspective of translational utility, genomics and DNA methylation profiling currently offer the clearest path to clinical implementation. Recurrent ESCC alterations such as TP53 and NOTCH1 mutations, CDKN2A deletions, and robust CpG methylation signatures can be adapted to targeted NGS or PCR-based assays compatible with existing clinical workflows. By contrast, proteomic and metabolomic biomarkers (e.g., PGK1 phosphorylation, tryptophan-pathway metabolites) exhibit platform-dependent variability and require MS expertise, limiting routine clinical adoption. Likewise, single-cell and spatial omics provide unparalleled mechanistic resolution (e.g., CST1⁺ CAFs, exhausted T-cell states) but currently function more as discovery tools than practical diagnostics. In addition, the lack of standardized analytical pipelines (e.g., for sample processing, tumor heterogeneity assessment, and data analysis), insufficient multicenter validation and collaboration, limitations in detection and diagnostic frameworks, and the high cost and technical complexity of integrating multi-omics data into routine clinical workflows remain major challenges.

To facilitate broader clinical translation in ESCC, future efforts should prioritize converting high-dimensional multi-omics discoveries into affordable, robust, and standardized assays. In the near term, realistic candidates for routine laboratory implementation include ESCC-specific DNA methylation panels, targeted sequencing panels covering actionable ESCC drivers (e.g., EGFR, HER2, VEGF/VEGFR, HGF/c-MET) and epigenetic regulators (e.g.,

DNA methyltransferases and histone-modification genes), as well as immune biomarkers (e.g., PD-1/PD-L1, CTLA4, TIM-3, LAG3, TIGIT) and selected protein markers (e.g., PGK1, CLK1, CSNK2A1). The development of automated, ESCC-tailored bioinformatics pipelines will further lower implementation barriers, especially for laboratories without dedicated computational expertise. Meanwhile, rigorous assessment of clinical utility remains essential: candidate metabolomic markers (e.g., creatine, α -tocopherol) and proposed therapeutic targets (e.g., PGK1, CLK1, CSNK2A1) require multicenter validation before adoption. Finally, multi-omics-defined ESCC subtypes, such as immune-modulated and NRF2-activated states, should be tested in prospective clinical trials to determine their value for treatment stratification, and future work integrating artificial intelligence with multi-omics data may further improve diagnostic accuracy and enable more precise therapeutic decision-making in ESCC.

Conclusions

Multi-omics investigations have substantially reshaped the understanding of ESCC by delineating its genomic instability, epigenetic deregulation, metabolic rewiring, and immune microenvironment heterogeneity, thereby revealing new biomarkers and therapeutic vulnerabilities. Yet, the translation of these insights into routine clinical practice remains limited, as many discoveries rely on resource-intensive platforms and lack cross-cohort validation. Future efforts should prioritize distilling complex multi-omics signals into robust, cost-effective assays compatible with standard diagnostic laboratories, supported by harmonized analytical pipelines and multicenter ESCC cohorts. Integrating genomic, methylation, and immune signatures into prospective clinical trials will be essential for defining molecular subtypes with predictive value. With continued refinement, multi-omics-driven approaches hold strong potential to advance early detection, personalized risk stratification, and the development of targeted therapies, ultimately improving clinical management and patient outcomes in ESCC.

Acknowledgements

We would like to acknowledge the assistance of BioRender.com in creating cartoon illustrations.

Abbreviations

ABCD1, ATP binding cassette subfamily D member 1; ADAM29, ADAM metallopeptidase domain 29; ADAMTS12, ADAM metallopeptidase with thrombospondin type 1 motif 12; ADGB, androglobin; ADH1B, alcohol dehydrogenase 1B (class I), beta polypeptide; AGR2, anterior gradient protein 2 homolog; AJUBA, ajuba LIM protein; AKAP9, A-kinase anchoring protein 9; AKR1C1, aldo-keto

reductase family 1 member C1; AKR1C2, aldo-keto reductase family 1 member C2; ALDH1A3, aldehyde dehydrogenase 1 family member A3; ALDH2, aldehyde dehydrogenase 2 family member; ALDH3A1, aldehyde dehydrogenase 3 family member A1; ALDH3B1, aldehyde dehydrogenase 3 family member B1; ALDH3B2, aldehyde dehydrogenase 3 family member B2; AMH, anti-Mullerian hormone; AMPK, protein kinase AMP-activated catalytic subunit alpha 1; ANO1, anoctamin 1; ANXA1, annexin A1; APC, APC regulator of WNT signaling pathway; ARAP2, ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2; AREG, amphiregulin; ARID1A, AT-rich interaction domain 1A; ASH1L, ASH1 like histone lysine methyltransferase; ASPA, aspartoacylase; ASXL1, ASXL transcriptional regulator 1; ATM, ATM serine/threonine kinase; ATP1B2, ATPase Na⁺/K⁺ transporting subunit beta 2; AXIN2, axin 2; B2M, beta-2-microglobulin; BAIAP2, BAR/IMD domain containing adaptor protein 2; BCL11B, BCL11 transcription factor B; BLM, RecQ-like DNA helicase BLM; BNC1, basonuclin zinc finger protein 1; BRAP, BRCA1 associated protein; BRCA1, BRCA1 DNA repair associated; BRCA2, BRCA2 DNA repair associated; BRIP1, BRCA1 interacting DNA helicase 1; C1orf226, chromosome 1 open reading frame 226; CASC9, cancer susceptibility 9; CASP1, caspase 1; CASP4, caspase 4; CASP8, caspase 8; CBX4, chromobox 4; CBX8, chromobox 8; CCND1, cyclin D1; CCNE1, cyclin E1; CD138, syndecan 1; CD274, CD274 molecule; CD276, CD276 molecule; CDC25A, cell division cycle 25A; CDC27, cell division cycle 27; CDCA5, cell division cycle associated 5; CDH1, cadherin 1; CDH10, cadherin 10; CDK4, cyclin-dependent kinase 4; CDK6, cyclin dependent kinase 6; CDKN1B, cyclin dependent kinase inhibitor 1B; CDKN2A, cyclin dependent kinase inhibitor 2A; CDKN2B, cyclin-dependent kinase 4 inhibitor B; CHAF1A, chromatin assembly factor 1 subunit A; CLCA4, chloride channel accessory 4; CLIC, chloride intracellular channel; CLIC3, chloride intracellular channel 3; CLIC4, chloride intracellular channel 4; CLK1, CDC like kinase 1; CNTNAP3, contactin associated protein family member 3; COL11A1, collagen type XI alpha 1 chain; COL14A1, collagen type XIV alpha 1 chain; COL1A1, collagen type I alpha 1 chain; COL1A2, collagen type I alpha 2 chain; COL3A1, collagen type III alpha 1 chain; CRCT1, cysteine rich C-terminal 1; CREBBP, CREB binding protein; CRISP3, cysteine rich secretory protein 3; CRNN, cornulin; CSF3R, colony stimulating factor 3; CSMD3, CUB and Sushi multiple domains 3; CSNK2A1, casein kinase 2 alpha 1; CTLA4, cytotoxic T-lymphocyte protein 4; CTTN, cortactin; CUL3, cullin 3; CXCL17, C-X-C motif chemokine 17; CYFIP2, cytoplasmic FMR1-interacting protein 2; CYP24A1, cytochrome P450 family 24 subfamily A member 1; CYP2A6, cytochrome P450 family 2 subfamily A member 6; DANCR, putative uncharacterized protein DANCR; DNAH5, dynein axonemal heavy chain 5; DOPEY1, protein DOP1A; DOPEY2,

DOP1 leucine zipper like protein B; DSC3, desmocollin 3; DSG3, desmoglein 3; DUXAP8, double homeobox A pseudogene 8; E2F2, E2F transcription factor 2; ECG2, serine peptidase inhibitor Kazal type 7; EGFL6, epidermal growth factor-like protein 6; EGFR, epidermal growth factor receptor; ELOA, elongin-A; EP300, E1A binding protein p300; EPB41L3, erythrocyte membrane protein band 4.1 like 3; ERBB2, erb-b2 receptor tyrosine kinase 2; ERBB4, erb-b2 receptor tyrosine kinase 4; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; FADD, Fas associated via death domain; FAK, focal adhesion kinase; FAM135B, protein FAM135B; FAM90A1, family with sequence similarity 90 member A1; FAT, protocadherin Fat; FAT1, FAT atypical cadherin 1; FAT2, FAT atypical cadherin 2; FAT3, FAT atypical cadherin 3; FAT4, FAT atypical cadherin 4; FBL, fibrillarin; FBXW7, F-box and WD repeat domain containing 7; FGFR, fibroblast growth factor receptor; FGFR1, fibroblast growth factor receptor 1; FGFR2, fibroblast growth factor receptor 2; FGFR3, fibroblast growth factor receptor 3; FHIT, fragile histidine triad diadenosine triphosphatase; FLG, filaggrin; FN1, fibronectin 1; FOXC2, forkhead box protein C2; FOXM1, forkhead box M1; FOXP3, forkhead box P3; FSCN1, fascin actin-bundling protein 1; FTO, FTO alpha-ketoglutarate dependent dioxygenase; GATA4, GATA binding protein 4; GATA6, GATA binding protein 6; GBP1, guanylate binding protein 1; GLUT1, solute carrier family 2, facilitated glucose transporter member 1; GMPS, guanosine monophosphate synthase; GNB4, G protein subunit beta 4; GPR116, adhesion G protein-coupled receptor F5; GPR68, G protein-coupled receptor 68; GPX3, Glutathione p

Author contributions

D.Y.Z. and X.Y.H. wrote the original review. D.Y.Z., X.Y.H. and Y.P.G. created all original figures. D.Y.Z., Y.P.G. and H.F.W. collected the data. Z.G.D. and K.D.L. revised the review and provided editorial assistance. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing interests.

Funding

This work was supported by grants from the National Natural Science Foundation of China (grant 82472998), the Central Plains Science and Technology Innovation Leading Talents (grant 224200510015), and the China Postdoctoral Science Foundation (grant 2021M692936).

Consent for publication

The authors agree with the publication.

References

- Agrawal N, Jiao Y, Bettgowda C et al. Comparative genomic analysis of esophageal adenocarcinoma and squamous cell carcinoma. *Cancer Discov* 2012;**2**:899–905.
- Akbari MR, Malekzadeh R, Shakeri R et al. Candidate gene association study of esophageal squamous cell carcinoma in a high-risk region in Iran. *Cancer Res* 2009;**69**:7994–8000.
- Alaei S, Sadeghi B, Najafi A et al. LncRNA and mRNA integration network reconstruction reveals novel key regulators in esophageal squamous-cell carcinoma. *Genomics* 2019;**111**:76–89.
- Aoyagi K, Minashi K, Igaki H et al. Artificially induced epithelial-mesenchymal transition in surgical subjects: its implications in clinical and basic cancer research. *PLoS One* 2011;**6**:e18196.
- Bandla S, Pennathur A, Luketich JD et al. Comparative genomics of esophageal adenocarcinoma and squamous cell carcinoma. *Ann Thorac Surg* 2012;**93**:1101–1106.
- Bazin J, Baerenfaller K, Gosai SJ et al. Global analysis of ribosome-associated noncoding RNAs unveils new modes of translational regulation. *Proc Natl Acad Sci U S A* 2017;**114**:E10018–E10027.
- Bi Y, Guo S, Xu X et al. Decreased ZNF750 promotes angiogenesis in a paracrine manner via activating DANCER/miR-4707-3p/FOXC2 axis in esophageal squamous cell carcinoma. *Cell Death Dis* 2020;**11**:296.
- Cancer Genome Atlas Research Network, Analysis Working Group: Asan University, BC Cancer Agency et al. Integrated genomic characterization of oesophageal carcinoma. *Nature* 2017; **541**:169–175.
- Cao S, Wang X, Liu X et al. Integrative analysis of angiogenesis-related long non-coding RNA and identification of a six-DEARlncRNA signature associated with prognosis and therapeutic response in esophageal squamous cell carcinoma. *Cancers (Basel)* 2022;**14**:4195.
- Cao W, Lee H, Wu W et al. Multi-faceted epigenetic dysregulation of gene expression promotes esophageal squamous cell carcinoma. *Nat Commun* 2020;**11**:3675.
- Cao W, Shi F, Wu L et al. Genome wide screening and characterization of long non-coding RNAs in esophageal cancer. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2014;**31**:587–590.
- Chang J, Tan W, Ling Z et al. Genomic analysis of oesophageal squamous-cell carcinoma identifies alcohol drinking-related mutation signature and genomic alterations. *Nat Commun* 2017;**8**:15290.
- Chen C, Zhao M, Yin N et al. Abnormal histone acetylation and methylation levels in esophageal squamous cell carcinomas. *Cancer Invest* 2011;**29**:548–556.
- Chen K, Li Y, Dai Y et al. Characterization of tumor suppressive function of cornulin in esophageal squamous cell carcinoma. *PLoS One* 2013;**8**:e68838.
- Chen SW, Zhou HF, Zhang HJ et al. The clinical significance and potential molecular mechanism of PTTG1 in esophageal squamous cell carcinoma. *Front Genet* 2020a;**11**:583085.
- Chen X, Cai S, Li B et al. MicroRNA21 regulates the biological behavior of esophageal squamous cell carcinoma by targeting RASA1. *Oncol Rep* 2019;**41**:1627–1637.
- Chen X, Zhao Y, Wang Y et al. Single-cell atlas of the esophageal squamous cell carcinoma immune ecosystem to predict immunotherapy response. *Signal Transduct Target Ther* 2025;**10**:348.
- Chen Y, Liao LD, Wu ZY et al. Identification of key genes by integrating DNA methylation and next-generation transcriptome sequencing for esophageal squamous cell carcinoma. *Aging (Albany NY)* 2020b;**12**:1332–1365.
- Chen YK, Tung CW, Lee, JY et al. Plasma matrix metalloproteinase 1 improves the detection and survival prediction of esophageal squamous cell carcinoma. *Sci Rep* 2016;**6**:30057.
- Chen Z, Gao Y, Huang X et al. Tissue-based metabolomics reveals metabolic biomarkers and potential therapeutic targets for esophageal squamous cell carcinoma. *J Pharm Biomed Anal* 2021a;**197**:113937.
- Chen Z, Huang X, Gao Y et al. Plasma-metabolite-based machine learning is a promising diagnostic approach for esophageal squamous cell carcinoma investigation. *J Pharm Anal* 2021b;**11**:505–514.
- Chen Z, Li, J, Tian L et al. MiRNA expression profile reveals a prognostic signature for esophageal squamous cell carcinoma. *Cancer Lett* 2014;**350**:34–42.
- Chen Z, Zhao M, Liang J et al. Dissecting the single-cell transcriptome network underlying esophagus non-malignant tissues and esophageal squamous cell carcinoma. *EBioMedicine* 2021c;**69**:103459.
- Cheng J, Jin H, Hou X et al. Disturbed tryptophan metabolism correlating to progression and metastasis of esophageal squamous cell carcinoma. *Biochem Biophys Res Commun* 2017;**486**:781–787.
- Cheng J, Liu Q, Jin H et al. Integrating transcriptome and metabolome variability to reveal pathogenesis of esophageal squamous cell carcinoma. *Biochim Biophys Acta Mol Basis Dis* 2021;**1867**:165966.
- Cheng J, Ma H, Yan M et al. Circ_0007624 suppresses the development of esophageal squamous cell carcinoma via targeting miR-224-5p/CPEB3 to inactivate the EGFR/PI3K/AKT signaling. *Cell Signal* 2022;**99**:110448.
- Cotton S, Ferreira D, Soares J et al. Target score-A proteomics data selection tool applied to esophageal cancer identifies GLUT1-Sialyl Tn glycoforms as biomarkers of cancer aggressiveness. *Int J Mol Sci* 2021;**22**:1664.
- Cui H, Zhou Y, Wang F et al. Characterization of somatic structural variations in 528 Chinese individuals with esophageal squamous cell carcinoma. *Nat Commun* 2022;**13**:6296.
- Cui S, Mcgrnahan N, Gao J et al. Tracking the evolution of esophageal squamous cell carcinoma under dynamic immune selection by multi-omics sequencing. *Nat Commun* 2023;**14**:892.
- Cui Y, Chen H, Xi R et al. Whole-genome sequencing of 508 patients identifies key molecular features associated with poor prognosis in esophageal squamous cell carcinoma. *Cell Res* 2020;**30**:902–913.
- Cui Y, Zhang C, Ma, S et al. RNA m⁶A demethylase FTO-mediated epigenetic up-regulation of LINC00022 promotes tumorigenesis in esophageal squamous cell carcinoma. *J Exp Clin Cancer Res* 2021;**40**:294.

- Dai W, Ko JMY, Choi SSA et al. Whole-exome sequencing reveals critical genes underlying metastasis in oesophageal squamous cell carcinoma. *J Pathol* 2017;**242**: 500–510.
- David R, Burgess A, Parker B et al. Transcriptome-wide mapping of RNA 5-methylcytosine in arabidopsis mRNAs and noncoding RNAs. *Plant Cell* 2017;**29**:445–460.
- Delaunay S, Frye M. RNA modifications regulating cell fate in cancer. *Nat Cell Biol* 2019;**21**:552–559.
- Ding J, Li, C, Cheng Y et al. Alterations of RNA splicing patterns in esophagus squamous cell carcinoma. *Cell Biosci* 2021;**11**:36.
- Dinh HQ, Pan F, Wang G et al. Integrated single-cell transcriptome analysis reveals heterogeneity of esophageal squamous cell carcinoma microenvironment. *Nat Commun* 2021;**12**:7335.
- Enzinger PC, Mayer RJ. Esophageal cancer. *N Engl J Med* 2003;**349**:2241–2252.
- Erkizan HV, Johnson K, Ghimbovschi S et al. African-American esophageal squamous cell carcinoma expression profile reveals dysregulation of stress response and detox networks. *BMC Cancer* 2017;**17**:426.
- Erkizan HV, Sukhadia S, Natarajan TG et al. Exome sequencing identifies novel somatic variants in African American esophageal squamous cell carcinoma. *Sci Rep* 2021;**11**:14814.
- Fan L, Cao Q, Liu J et al. Circular RNA profiling and its potential for esophageal squamous cell cancer diagnosis and prognosis. *Mol Cancer* 2019;**18**:16.
- Fu MG, Li S, Yu TT et al. Differential expression of miR-195 in esophageal squamous cell carcinoma and miR-195 expression inhibits tumor cell proliferation and invasion by targeting of Cdc42. *FEBS Lett* 2013;**587**:3471–3479.
- Fujigaki S, Nishiumi S, Kobayashi T et al. Identification of serum biomarkers of chemoradiosensitivity in esophageal cancer via the targeted metabolomics approach. *Biomark Med* 2018;**12**:827–840.
- Fujihara S, Kobara H, Nishiyama N et al. MicroRNA expression profiles in superficial esophageal squamous cell carcinoma before endoscopic submucosal dissection: a pilot study. *Int J Mol Sci* 2021;**22**:4789.
- Gao Y, Shen L, Dong T et al. An N-glycoproteomic site-mapping analysis reveals glycoprotein alterations in esophageal squamous cell carcinoma. *J Transl Med* 2022;**20**:285.
- Gao YB, Chen Z-L, Li, J-G et al. Genetic landscape of esophageal squamous cell carcinoma. *Nat Genet* 2014;**46**:1097–1102.
- Gerashchenko TS, Denisov EV, Litviakov NV et al. Intratumor heterogeneity: nature and biological significance. *Biochemistry (Mosc)* 2013;**78**:1201–1215.
- Gu, L, Sang Y, Nan X et al. circCYP24A1 facilitates esophageal squamous cell carcinoma progression through binding PKM2 to regulate NF- κ B-induced CCL5 secretion. *Mol Cancer* 2022;**21**:217.
- Guagnano V, Kauffmann A, Wöhrle S et al. FGFR genetic alterations predict for sensitivity to NVP-BGJ398, a selective pan-FGFR inhibitor. *Cancer Discov* 2012;**2**:1118–1133.
- Guo J, Huang J, Zhou Y et al. Germline and somatic variations influence the somatic mutational signatures of esophageal squamous cell carcinomas in a Chinese population. *BMC Genomics* 2018;**19**:538.
- Guo Y, Chen Z, Zhang L et al. Distinctive microRNA profiles relating to patient survival in esophageal squamous cell carcinoma. *Cancer Res* 2008;**68**:26–33.
- Guo Z, Pan F, Peng L et al. Systematic proteome and lysine succinylome analysis reveals enhanced cell migration by hyposuccinylation in esophageal squamous cell carcinoma. *Mol Cell Proteomics* 2021;**20**:100053.
- Han, BA, Yang XP, Hosseini DK et al. Identification of candidate aberrantly methylated and differentially expressed genes in esophageal squamous cell carcinoma. *Sci Rep* 2020;**10**:9735.
- Hao JJ, Lin DC, Dinh HQ et al. Spatial intratumoral heterogeneity and temporal clonal evolution in esophageal squamous cell carcinoma. *Nat Genet* 2016;**48**:1500–1507.
- He R, Wang J, Ye K et al. Reduced miR-203 predicts metastasis and poor survival in esophageal carcinoma. *Aging (Albany NY)* 2019;**11**:12114–12130.
- Hirata H, Niida A, Kakiuchi N et al. The evolving genomic landscape of esophageal squamous cell carcinoma under chemoradiotherapy. *Cancer Res* 2021;**81**:4926–4938.
- Hoshimoto S, Takeuchi H, Ono S et al. Genome-wide hypomethylation and specific tumor-related gene hypermethylation are associated with esophageal squamous cell carcinoma outcome. *J Thorac Oncol* 2015;**10**:509–517.
- Hu N, Clifford RJ, Yang HH et al. Genome wide analysis of DNA copy number neutral loss of heterozygosity (CNNLOH) and its relation to gene expression in esophageal squamous cell carcinoma. *BMC Genomics* 2010;**11**:576.
- Hu, N, Kadota M, Liu H et al. Genomic landscape of somatic alterations in esophageal squamous cell carcinoma and gastric cancer. *Cancer Res* 2016;**76**:1714–1723.
- Hu N, Wang C, Clifford RJ et al. Integrative genomics analysis of genes with biallelic loss and its relation to the expression of mRNA and micro-RNA in esophageal squamous cell carcinoma. *BMC Genomics* 2015;**16**:732.
- Hu X, Wu D, He, X et al. circGSK3 β promotes metastasis in esophageal squamous cell carcinoma by augmenting β -catenin signaling. *Mol Cancer* 2019;**18**:160.
- Huang GW, Xue YJ, Wu ZY et al. A three-lncRNA signature predicts overall survival and disease-free survival in patients with esophageal squamous cell carcinoma. *BMC Cancer* 2018;**18**:147.
- Ibuki Y, Nishiyama Y, Tsutani Y et al. Circulating microRNA/isomiRs as novel biomarkers of esophageal squamous cell carcinoma. *PLoS One* 2020;**15**:e0231116.
- Jang HJ, Lee HS, Burt BM et al. Integrated genomic analysis of recurrence-associated small non-coding RNAs in oesophageal cancer. *Gut* 2017;**66**:215–225.
- Jiao Z, Yu A, Rong W et al. Five-lncRNA signature in plasma exosomes serves as diagnostic biomarker for esophageal squamous cell carcinoma. *Aging (Albany NY)* 2020;**12**:15002–15010.
- Jin H, Qiao F, Chen L et al. Serum metabolomic signatures of lymph node metastasis of esophageal squamous cell carcinoma. *J Proteome Res* 2014;**13**:4091–4103.

- Jin X, Liu L, Wu J et al. A multi-omics study delineates new molecular features and therapeutic targets for esophageal squamous cell carcinoma. *Clin Transl Med* 2021;**11**:e538.
- Kang X, Chen K, Li Y et al. Personalized targeted therapy for esophageal squamous cell carcinoma. *World J Gastroenterol* 2015;**21**:7648–7658.
- Kanwal R, Gupta S. Epigenetic modifications in cancer. *Clin Genet* 2012;**81**:303–311.
- Kearns B, Mckell A, Steveson I et al. ARID1A and its impact across the hallmarks of cancer. *Int J Mol Sci* 2025;**26**:4644.
- Khoddami V, Cairns BR. Identification of direct targets and modified bases of RNA cytosine methyltransferases. *Nat Biotechnol* 2013;**31**:458–464.
- Komatsu S, Ichikawa D, Hirajima S et al. Plasma microRNA profiles: identification of miR-25 as a novel diagnostic and monitoring biomarker in oesophageal squamous cell carcinoma. *Br J Cancer* 2014;**111**:1614–1624.
- Kong KL, Kwong DL, Chan TH et al. MicroRNA-375 inhibits tumour growth and metastasis in oesophageal squamous cell carcinoma through repressing insulin-like growth factor 1 receptor. *Gut* 2012;**61**:33–42.
- Kristensen LS, Andersen MS, Stagsted LVW et al. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet*, 2019;**20**:675–691.
- Lee JJ, Natsuzaka M, Ohashi S et al. Hypoxia activates the cyclooxygenase-2-prostaglandin E synthase axis. *Carcinogenesis* 2010;**31**:427–434.
- Li B, Zhang Y, Miao L et al. Esophagectomy with three-field versus two-field lymphadenectomy for middle and lower thoracic esophageal cancer: long-term outcomes of a randomized clinical trial. *J Thorac Oncol* 2021a;**16**:310–317.
- Li CY, Zhang WW, Xiang JL et al. Identification of microRNAs as novel biomarkers for esophageal squamous cell carcinoma: a study based on The Cancer Genome Atlas (TCGA) and bioinformatics. *Chin Med J (Engl)* 2019a;**132**:2213–2222.
- Li D, Zhang L, Liu Y et al. Specific DNA methylation markers in the diagnosis and prognosis of esophageal cancer. *Aging (Albany NY)* 2019b;**11**:11640–11658.
- Li J, Chen Z, Tian L et al. LncRNA profile study reveals a three-lncRNA signature associated with the survival of patients with oesophageal squamous cell carcinoma. *Gut* 2014a;**63**:1700–1710.
- Li K, Lin Y, Zhou Y et al. Salivary extracellular microRNAs for early detection and prognostication of esophageal cancer: a clinical study. *Gastroenterology* 2023a;**165**:932–945 e9.
- Li L, Jiang D, Zhang Q et al. Integrative proteogenomic characterization of early esophageal cancer. *Nat Commun* 2023b;**14**:1666.
- Li L, Ma G, Jing C et al. Guanylate-binding protein 1 (GBP1) promotes lymph node metastasis in human esophageal squamous cell carcinoma. *Discov Med* 2015;**20**:369–378.
- Li M, Wang K, Pang Y et al. Secreted phosphoprotein 1 (SPP1) and fibronectin 1 (FN1) are associated with progression and prognosis of esophageal cancer as identified by integrated expression profiles analysis. *Med Sci Monit* 2020a;**26**:e920355.
- Li M, Zhang Z, Wang Q et al. Integrated cohort of esophageal squamous cell cancer reveals genomic features underlying clinical characteristics. *Nat Commun* 2022;**13**:5268.
- Li M, Zhao J, Li, X et al. HiFreSP: a novel high-frequency sub-pathway mining approach to identify robust prognostic gene signatures. *Brief Bioinform* 2020b;**21**:1411–1424.
- Li SQ, Li F, Xiao Y et al. Comparison of long noncoding RNAs, microRNAs and messenger RNAs involved in initiation and progression of esophageal squamous cell carcinoma. *Mol Med Rep* 2014b;**10**:652–662.
- Li W, Liu J, Zhao H Identification of a nomogram based on long non-coding RNA to improve prognosis prediction of esophageal squamous cell carcinoma. *Aging (Albany NY)* 2020c;**12**:1512–1526.
- Li X, Xiao X, Chang R et al. Comprehensive bioinformatics analysis identifies lncRNA HCG22 as a migration inhibitor in esophageal squamous cell carcinoma. *J Cell Biochem* 2020d;**121**:468–481.
- Li X, Zhao L, Wei M et al. Serum metabolomics analysis for the progression of esophageal squamous cell carcinoma. *J Cancer* 2021b;**12**:3190–3197.
- Li X, Zhou F, Jiang C et al. Identification of a DNA methylome profile of esophageal squamous cell carcinoma and potential plasma epigenetic biomarkers for early diagnosis. *PLoS One* 2014c;**9**:e103162.
- Li XC, Wang MY, Yang M et al. A mutational signature associated with alcohol consumption and prognostically significantly mutated driver genes in esophageal squamous cell carcinoma. *Ann Oncol* 2018;**29**:938–944.
- Li Y, Yang B, Ma Y et al. Phosphoproteomics reveals therapeutic targets of esophageal squamous cell carcinoma. *Signal Transduct Target Ther* 2021c;**6**:381.
- Liang F, Luo Q, Han H et al. Long noncoding RNA LINC01088 inhibits esophageal squamous cell carcinoma progression by targeting the NPM1-HDM2-p53 axis. *Acta Biochim Biophys Sin (Shanghai)* 2023;**55**:367–381.
- Liang Y, Mao Q, Wang L et al. CircIMMP2L promotes esophageal squamous cell carcinoma malignant progression via CtBP1 nuclear retention dependent epigenetic modification. *Clin Transl Med* 2021;**11**:e519.
- Liang Y, Wu Y, Chen X et al. A novel long noncoding RNA linc00460 up-regulated by CBP/P300 promotes carcinogenesis in esophageal squamous cell carcinoma. *Biosci Rep* 2017;**37**:BSR20171019.
- Lima SC, Hernández-Vargas H, Simão T et al. Identification of a DNA methylome signature of esophageal squamous cell carcinoma and potential epigenetic biomarkers. *Epigenetics* 2011;**6**:1217–1227.
- Lin C, Zhang S, Wang Y et al. Functional role of a novel long noncoding RNA TTN-AS1 in esophageal squamous cell carcinoma progression and metastasis. *Clin Cancer Res* 2018;**24**:486–498.
- Lin DC, Hao JJ, Nagata Y et al. Genomic and molecular characterization of esophageal squamous cell carcinoma. *Nat Genet* 2014;**46**:467–473.
- Lin L, Lin DC. Biological significance of tumor heterogeneity in esophageal squamous cell carcinoma. *Cancers (Basel)* 2019;**11**:1156.

- Liu G, Zhao Y, Chen H et al. Analysis of differentially expressed genes in a Chinese cohort of esophageal squamous cell carcinoma. *J Cancer* 2020a;**11**:3783–3793.
- Liu L, Wu J, Shi M et al. New metabolic alterations and a predictive marker pipelicolic acid in sera for esophageal squamous cell carcinoma. *Genomics, Proteomics & Bioinformatics*, 2022a;**20**:670–687.
- Liu M, An H, Zhang Y et al. Molecular analysis of Chinese oesophageal squamous cell carcinoma identifies novel subtypes associated with distinct clinical outcomes. *EBioMedicine* 2020b;**57**:102831.
- Liu QW, Ruan HJ, Chao WX et al. N-linked glycoproteomic profiling in esophageal squamous cell carcinoma. *World J Gastroenterol* 2022b;**28**:3869–3885.
- Liu R, Gu J, Jiang P et al. DNMT1-microRNA126 epigenetic circuit contributes to esophageal squamous cell carcinoma growth via ADAM9-EGFR-AKT signaling. *Clin Cancer Res* 2015;**21**:854–863.
- Liu R, Peng Y, Li X et al. Identification of plasma metabolomic profiling for diagnosis of esophageal squamous-cell carcinoma using an UPLC/TOF/MS platform. *Int J Mol Sci* 2013;**14**:8899–8911.
- Liu W, Snell JM, Jeck WR et al. Subtyping sub-Saharan esophageal squamous cell carcinoma by comprehensive molecular analysis. *JCI Insight* 2016;**1**:e88755.
- Liu W, Xie L, He YH et al. Large-scale and high-resolution mass spectrometry-based proteomics profiling defines molecular subtypes of esophageal cancer for therapeutic targeting. *Nat Commun* 2021a;**12**:4961.
- Liu W, Zhang Y, Chen M et al. A genome-wide analysis of long noncoding RNA profile identifies differentially expressed lncRNAs associated with esophageal cancer. *Cancer Med* 2018;**7**:4181–4189.
- Liu, X, Zhao S, Wang K et al. Spatial transcriptomics analysis of esophageal squamous precancerous lesions and their progression to esophageal cancer. *Nat Commun* 2023a;**14**:4779.
- Liu Y-Y, Yang Z-X, Ma L-M et al. H-NMR spectroscopy identifies potential biomarkers in serum metabolomic signatures for early stage esophageal squamous cell carcinoma. *PeerJ* 2019;**7**:e8151.
- Liu Z, Gu S, Wu K et al. CircRNA-DOPEY2 enhances the chemosensitivity of esophageal cancer cells by inhibiting CPEB4-mediated mcl-1 translation. *J Exp Clin Cancer Res* 2021b;**40**:361.
- Liu Z, Zhao Y, Kong P et al. Integrated multi-omics profiling yields a clinically relevant molecular classification for esophageal squamous cell carcinoma. *Cancer Cell* 2023b;**41**:181–195.e9.
- Lu T, Chen D, Wang Y et al. Identification of DNA methylation-driven genes in esophageal squamous cell carcinoma: a study based on The Cancer Genome Atlas. *Cancer Cell International*, 2019;**19**:52.
- Ma H, Hasim A, Mamtimin B et al. Plasma free amino acid profiling of esophageal cancer using high-performance liquid chromatography spectroscopy. *World J Gastroenterol* 2014;**20**:8653–8659.
- Ma J, Han LN, Song JR et al. Long noncoding RNA LINC01234 silencing exerts an anti-oncogenic effect in esophageal cancer cells through microRNA-193a-5p-mediated CCNE1 downregulation. *Cell Oncol (Dordr)* 2020;**43**:377–394.
- Ma J, Xiao Y, Tian B et al. Genome-wide analyses of long non-coding RNA expression profiles and functional network analysis in esophageal squamous cell carcinoma. *Sci Rep* 2019;**9**:9162.
- Ma K, Cao B, Guo M. The detective, prognostic, and predictive value of DNA methylation in human esophageal squamous cell carcinoma. *Clin Epigenetics* 2016;**8**:43.
- Mangalaparthy KK, Patel K, Khan AA et al. Mutational landscape of esophageal squamous cell carcinoma in an Indian cohort. *Front Oncol* 2020;**10**:1457.
- Mao Y, Fu Z, Zhang Y et al. A seven-lncRNA signature predicts overall survival in esophageal squamous cell carcinoma. *Sci Rep* 2018;**8**:8823.
- Mao Y, Fu Z, Zhang Y et al. A six-microRNA risk score model predicts prognosis in esophageal squamous cell carcinoma. *J Cell Physiol* 2019;**234**:6810–6819.
- Mathé EA, Nguyen GH, Bowman ED et al. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. *Clin Cancer Res* 2009;**15**:6192–6200.
- Matsui K, Kawakubo H, Matsuda S et al. Clinical features of recurrence pattern with lung metastasis after radical esophagectomy for thoracic esophageal cancer. *World J Surg* 2022;**46**:2270–2279.
- Mei LL, Qiu YT, Huang MB et al. MiR-99a suppresses proliferation, migration and invasion of esophageal squamous cell carcinoma cells through inhibiting the IGF1R signaling pathway. *Cancer Biomark* 2017a;**20**:527–537.
- Mei LL, Wang WJ, Qiu YT et al. miR-145-5p suppresses tumor cell migration, invasion and epithelial to mesenchymal transition by regulating the Sp1/NF- κ B signaling pathway in esophageal squamous cell carcinoma. *Int J Mol Sci* 2017b;**18**:1833.
- Meng H, Wang K, Chen X et al. MicroRNA-330-3p functions as an oncogene in human esophageal cancer by targeting programmed cell death 4. *Am J Cancer Res* 2015;**5**:1062–1075.
- Meng X, Chen X, Lu P et al. miR-202 promotes cell apoptosis in esophageal squamous cell carcinoma by targeting HSF2. *Oncol Res* 2017;**25**:215–223.
- Michaylira CZ, Wong GS, Miller CG et al. Periostin, a cell adhesion molecule, facilitates invasion in the tumor microenvironment and annotates a novel tumor-invasive signature in esophageal cancer. *Cancer Res*, 2010;**70**:5281–5292.
- Min Q, Wang Y, Wu Q et al. Genomic and epigenomic evolution of acquired resistance to combination therapy in esophageal squamous cell carcinoma. *JCI Insight* 2021;**6**:e150203.
- Ming XY, Zhang X, Cao TT et al. RHCG suppresses tumorigenicity and metastasis in esophageal squamous cell carcinoma via inhibiting NF- κ B signaling and MMP1 expression. *Theranostics* 2018;**8**:185–198.
- Mir SA, Rajagopalan P, Jain AP et al. LC-MS-based serum metabolomic analysis reveals dysregulation of

- phosphatidylcholines in esophageal squamous cell carcinoma. *J Proteomics* 2015;**127**:96–102.
- Miyoshi J, Zhu Z, Luo A et al. A microRNA-based liquid biopsy signature for the early detection of esophageal squamous cell carcinoma: a retrospective, prospective and multi-center study. *Mol Cancer* 2022;**21**:44.
- Morgan E, Soerjomataram I, Rumgay H et al. The global landscape of esophageal squamous cell carcinoma and esophageal adenocarcinoma incidence and mortality in 2020 and projections to 2040: new estimates from GLOBOCAN 2020. *Gastroenterology* 2022;**163**:649–658.e2.
- Munari FF, DOS Santos W, Evangelista AF et al. Profile of esophageal squamous cell carcinoma mutations in Brazilian patients. *Sci Rep* 2021;**11**:20596.
- Nakazato H, Takeshima H, Kishino T et al. Early-stage induction of SWI/SNF mutations during esophageal squamous cell carcinogenesis. *PLoS One* 2016;**11**:e0147372.
- Nicolau-Neto P, Da Costa NM, De Souza Santos PT et al. Esophageal squamous cell carcinoma transcriptome reveals the effect of FOXM1 on patient outcome through novel PIK3R3 mediated activation of PI3K signaling pathway. *Oncotarget* 2018;**9**:16634–16647.
- Ning Z, Zhu X, Jiang Y et al. Integrin-Linked kinase is involved in the proliferation and invasion of esophageal squamous cell carcinoma. *J Cancer* 2020;**11**:324–333.
- Nishimori T, Tomonaga T, Matsushita K et al. Proteomic analysis of primary esophageal squamous cell carcinoma reveals downregulation of a cell adhesion protein, periplakin. *Proteomics*, 2006;**6**:1011–1018.
- Nishiumi S, Fujigaki S, Kobayashi T et al. Metabolomics-based discovery of serum biomarkers to predict the side-effects of neoadjuvant chemoradiotherapy for esophageal squamous cell carcinoma. *Anticancer Res* 2019;**39**:519–526.
- Niu R, Zhao F, Dong Z et al. A stratification system of ferroptosis and iron-metabolism related lncRNAs guides the prediction of the survival of patients with esophageal squamous cell carcinoma. *Front Oncol* 2022;**12**:1010074.
- Okumura T, Kojima H, Miwa T et al. The expression of microRNA 574-3p as a predictor of postoperative outcome in patients with esophageal squamous cell carcinoma. *World J Surg Oncol* 2016;**14**:228.
- Onozato Y, Sasaki Y, Abe Y et al. Novel genomic alteration in superficial esophageal squamous cell neoplasms in non-smoker non-drinker females. *Sci Rep* 2021;**11**:20150.
- Ouyang T, Ma C, Zhao Y et al. 1 H NMR-based metabolomics of paired tissue, serum and urine samples reveals an optimized panel of biofluids metabolic biomarkers for esophageal cancer. *Front Oncol* 2023;**13**:1082841.
- Pan H, Wu Z, Zhang H et al. Identification and validation of IgG N-glycosylation biomarkers of esophageal carcinoma. *Front Immunol* 2023;**14**:981861.
- Pan Z, Lin J, Wu D et al. Hsa_circ_0006948 enhances cancer progression and epithelial-mesenchymal transition through the miR-490-3p/HMGA2 axis in esophageal squamous cell carcinoma. *Aging (Albany NY)* 2019;**11**:11937–11954.
- Pan Z, Mao W, Bao Y et al. The long noncoding RNA CASC9 regulates migration and invasion in esophageal cancer. *Cancer Med* 2016;**5**:2442–2447.
- Pang J, Pan H, Yang C et al. Prognostic value of immune-related multi-lncRNA signatures associated with tumor micro-environment in esophageal cancer. *Front Genet* 2021;**12**:722601.
- Pawar H, Kashyap MK, Sahasrabudhe NA et al. Quantitative tissue proteomics of esophageal squamous cell carcinoma for novel biomarker discovery. *Cancer Biol Ther* 2011;**12**:510–522.
- Pectasides E. Immune checkpoint blockade in esophageal squamous cell carcinoma: is it ready for prime time? *J Thorac Dis* 2018;**10**:1276–1279.
- Pei X, Liu Z, Tang L et al. Single-cell multi-omic and spatial profiling of esophageal squamous cell carcinoma reveals the immunosuppressive role of GPR116(+) pericytes in cancer metastasis. *Nat Genet* 2025;**57**:2494–2508.
- Peng H, Wang S, Pang L et al. Comprehensive bioinformation analysis of methylated and differentially expressed genes in esophageal squamous cell carcinoma. *Mol Omics* 2019;**15**:88–100.
- Peng X, Zhou Y, Chen Y et al. Reduced LINC00551 expression promotes proliferation and invasion of esophageal squamous cancer by increase in HSP27 phosphorylation. *J Cell Physiol* 2021;**236**:1418–1431.
- Pennathur A, Gibson MK, Jobe BA et al. Oesophageal carcinoma. *Lancet* 2013;**381**:400–412.
- Phatak P, Byrnes KA, Mansour D et al. Overexpression of miR-214-3p in esophageal squamous cancer cells enhances sensitivity to cisplatin by targeting survivin directly and indirectly through CUG-BP1. *Oncogene* 2016;**35**:2087–2097.
- Pohl H, Wrobel K, Bojarski C et al. Risk factors in the development of esophageal adenocarcinoma. *Am J Gastroenterol* 2013;**108**:200–207.
- Pu Y, Lu X, Yang X et al. Estimating the prognosis of esophageal squamous cell carcinoma based on The Cancer Genome Atlas (TCGA) of m6A methylation-associated genes. *J Gastrointest Oncol* 2022;**13**:1–12.
- Qin HD, Liao XY, Chen YB et al. Genomic characterization of esophageal squamous cell carcinoma reveals critical genes underlying tumorigenesis and poor prognosis. *Am J Hum Genet* 2016;**98**:709–727.
- Quante M, Graham TA, Jansen M. Insights into the pathophysiology of esophageal adenocarcinoma. *Gastroenterology* 2018;**154**:406–420.
- Rustgi AK, EL-Serag HB. Esophageal carcinoma. *N Engl J Med* 2014;**371**:2499–2509.
- Sawada G, Niida A, Hirata H et al. An integrative analysis to identify driver genes in esophageal squamous cell carcinoma. *PLoS One* 2015;**10**:e0139808.
- Sawada G, Niida A, Uchi R et al. Genomic landscape of esophageal squamous cell carcinoma in a Japanese population. *Gastroenterology* 2016;**150**:1171–1182.
- Sharma U, Barwal TS, Khandelwal A et al. Circulating long non-coding RNAs LINC00324 and LOC100507053 as

- potential liquid biopsy markers for esophageal squamous cell carcinoma: a pilot study. *Front Oncol* 2022;**12**:823953.
- Shen Y, Ding Y, Ma Q et al. Identification of novel circulating miRNA biomarkers for the diagnosis of esophageal squamous cell carcinoma and squamous dysplasia. *Cancer Epidemiol Biomarkers Prev* 2019;**28**:1212–1220.
- Shen Y, Shao Y, Niu C et al. Systematic identification of circRNA-miRNA-mRNA regulatory network in esophageal squamous cell carcinoma. *Front Genet* 2021a;**12**:580390.
- Shen Z, Chen M, Luo F et al. Identification of key genes and pathways associated with paclitaxel resistance in esophageal squamous cell carcinoma based on bioinformatics analysis. *Front Genet* 2021b;**12**:671639.
- Shi P, Sun J, He B et al. Profiles of differentially expressed circRNAs in esophageal and breast cancer. *Cancer Manag Res* 2018;**10**:2207–2221.
- Shi Y, Fang N, Li Y et al. Circular RNA LPAR3 sponges microRNA-198 to facilitate esophageal cancer migration, invasion, and metastasis. *Cancer Sci* 2020;**111**:2824–2836.
- Shi Y, Guo Z, Fang N et al. Hsa_circ_0006168 sponges miR-100 and regulates mTOR to promote the proliferation, migration and invasion of esophageal squamous cell carcinoma. *Biomed Pharmacother* 2019;**117**:109151.
- Shu J, MA J, Ren X et al. The abnormal glycoproteins of salivary glycoproteins in esophageal squamous cell carcinoma patients. *Front Chem* 2021;**9**:637730.
- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016;**66**:7–30.
- Singh S, Bano A, Saraya A et al. iTRAQ-based analysis for the identification of MARCH8 targets in human esophageal squamous cell carcinoma. *J Proteomics* 2021;**236**:104125.
- Singh V, Singh LC, Vasudevan M et al. Esophageal cancer epigenomics and integrative analysis of genome-wide methylation and expression in high risk northeast Indian population. *OMICS* 2015;**19**:688–699.
- Song Y, Li L, Ou Y et al. Identification of genomic alterations in oesophageal squamous cell cancer. *Nature* 2014;**509**:91–95.
- Song Y, Zhu S, Zhang N et al. Blood circulating miRNA pairs as a robust signature for early detection of esophageal cancer. *Front Oncol* 2021;**11**:723779.
- Squires JE, Patel HR, Nousch M et al. Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA. *Nucleic Acids Res* 2012;**40**:5023–5033.
- Su H, Hu N, Yang HH et al. Global gene expression profiling and validation in esophageal squamous cell carcinoma and its association with clinical phenotypes. *Clin Cancer Res* 2011;**17**:2955–2966.
- Su J, Wu G, Ye Y et al. NSUN2-mediated RNA 5-methylcytosine promotes esophageal squamous cell carcinoma progression via LIN28B-dependent GRB2 mRNA stabilization. *Oncogene* 2021;**40**:5814–5828.
- Sudo K, Kato K, Matsuzaki J et al. Development and validation of an esophageal squamous cell carcinoma detection model by large-scale microRNA profiling. *JAMA Netw Open* 2019;**2**:e194573.
- Sun C, Li T, Song X et al. Spatially resolved metabolomics to discover tumor-associated metabolic alterations. *Proc Natl Acad Sci U S A* 2019;**116**:52–57.
- Sun Q, Liu R, Zhang H et al. Lipidomic and transcriptomic analysis and its therapeutic implications in Chinese Kazakh patients with esophageal squamous cell carcinoma. *BMC Cancer* 2025;**25**:1696.
- Sun Q, Zong L, Zhang H et al. A 10microRNA prognosis scoring system in esophageal squamous cell carcinoma constructed using bioinformatic methods. *Mol Med Rep* 2018;**17**:5222–5228.
- Tabassum DP, Polyak K Tumorigenesis: it takes a village. *Nat Rev Cancer* 2015;**15**:473–483.
- Taccioli C, Garofalo M, Chen H et al. Repression of esophageal neoplasia and inflammatory signaling by anti-miR-31 delivery in vivo. *J Natl Cancer Inst* 2015;**107**:djv220.
- Takahashi T, Matsuda Y, Yamashita S et al. Estimation of the fraction of cancer cells in a tumor DNA sample using DNA methylation. *PLoS One* 2013;**8**:e82302.
- Takemoto A, Tanimoto K, Mori S et al. Integrative genome-wide analyses reveal the transcriptional aberrations in Japanese esophageal squamous cell carcinoma. *Cancer Sci* 2021;**112**:4377–4392.
- Takeshita N, Hoshino I, Mori M et al. Serum microRNA expression profile: miR-1246 as a novel diagnostic and prognostic biomarker for oesophageal squamous cell carcinoma. *Br J Cancer* 2013;**108**:644–652.
- Talukdar FR, Soares Lima SC, Khoeiry R et al. Genome-wide DNA methylation profiling of esophageal squamous cell carcinoma from global high-incidence regions identifies crucial genes and potential cancer markers. *Cancer Res* 2021;**81**:2612–2624.
- Tanaka Y, Aoyagi K, Minashi K et al. Discovery of a good responder subtype of esophageal squamous cell carcinoma with cytotoxic T-lymphocyte signatures activated by chemoradiotherapy. *PLoS One* 2015;**10**:e0143804.
- Testa U, Castelli G, Pelosi E. Esophageal cancer: genomic and molecular characterization, stem cell compartment and clonal evolution. *Medicines (Basel)* 2017;**4**:67.
- Tian L, Yang L, Zheng W et al. RNA sequencing of exosomes revealed differentially expressed long noncoding RNAs in early-stage esophageal squamous cell carcinoma and benign esophagitis. *Epigenomics* 2020;**12**:525–541.
- Toh Y, Egashira A, Yamamoto M. Epigenetic alterations and their clinical implications in esophageal squamous cell carcinoma. *Gen Thorac Cardiovasc Surg* 2013;**61**:262–269.
- Tong M, Chan KW, Bao JY et al. Rab25 is a tumor suppressor gene with antiangiogenic and anti-invasive activities in esophageal squamous cell carcinoma. *Cancer Res* 2012;**72**:6024–6035.
- Uemura N, Nakanishi Y, Kato H et al. Transglutaminase 3 as a prognostic biomarker in esophageal cancer revealed by proteomics. *Int J Cancer* 2009;**124**:2106–2115.
- Urabe F, Matsuzaki J, Yamamoto Y et al. Large-scale circulating microRNA profiling for the liquid biopsy of prostate cancer. *Clin Cancer Res* 2019a;**25**:3016–3025.

- Urabe Y, Kagemoto K, Hayes CN *et al.* Genomic characterization of early-stage esophageal squamous cell carcinoma in a Japanese population. *Oncotarget* 2019b;**10**:4139–4148.
- Usuba W, Urabe F, Yamamoto Y *et al.* Circulating miRNA panels for specific and early detection in bladder cancer. *Cancer Sci* 2019;**110**:408–419.
- Visvader JE. Cells of origin in cancer. *Nature* 2011;**469**:314–322.
- Wang C, Zhou M, Zhu P *et al.* IGF2BP2-induced circRUNX1 facilitates the growth and metastasis of esophageal squamous cell carcinoma through miR-449b-5p/FOXP3 axis. *J Exp Clin Cancer Res* 2022a;**41**:347.
- Wang D, Dai J, Suo C *et al.* Molecular subtyping of esophageal squamous cell carcinoma by large-scale transcriptional profiling: characterization, therapeutic targets, and prognostic value. *Front Genet* 2022b;**13**:1033214.
- Wang F, Zhang C, Cheng H *et al.* TGF- β -induced PLEK2 promotes metastasis and chemoresistance in oesophageal squamous cell carcinoma by regulating LCN2. *Cell Death Dis* 2021a;**12**:901.
- Wang H, Song X, Wang Y *et al.* CircCNTNAP3-TP53-positive feedback loop suppresses malignant progression of esophageal squamous cell carcinoma. *Cell Death Dis* 2020a;**11**:1010.
- Wang J, Luo FF, Huang TJ *et al.* The upregulated expression of RFC4 and GMPS mediated by DNA copy number alteration is associated with the early diagnosis and immune escape of ESCC based on a bioinformatic analysis. *Aging (Albany NY)* 2021b;**13**:21758–21777.
- Wang J, Yu P, Luo J *et al.* Transcriptomic and microRNA expression profiles identify biomarkers for predicting neo-chemoradiotherapy response in esophageal squamous cell carcinomas (ESCC). *Front Pharmacol* 2021c;**12**:626972.
- Wang K, Johnson A, Ali SM *et al.* Comprehensive genomic profiling of advanced esophageal squamous cell carcinomas and esophageal adenocarcinomas reveals similarities and differences. *Oncologist* 2015;**20**:1132–1139.
- Wang L, Liu G, Bolor-Erdene E *et al.* Identification of KIF4A as a prognostic biomarker for esophageal squamous cell carcinoma. *Aging (Albany NY)* 2021d;**13**:24050–24070.
- Wang PP, Song X, Zhao XK *et al.* Serum metabolomic profiling reveals biomarkers for early detection and prognosis of esophageal squamous cell carcinoma. *Front Oncol* 2022c;**12**:790933.
- Wang Q, Ma C, Kemmner W. Wdr66 is a novel marker for risk stratification and involved in epithelial-mesenchymal transition of esophageal squamous cell carcinoma. *BMC Cancer* 2013;**13**:137.
- Wang W, Wei C, Li P *et al.* Integrative analysis of mRNA and lncRNA profiles identified pathogenetic lncRNAs in esophageal squamous cell carcinoma. *Gene* 2018;**661**:169–175.
- Wang W, Zhu D, Zhao Z *et al.* RNA sequencing reveals the expression profiles of circRNA and identifies a four-circRNA signature acts as a prognostic marker in esophageal squamous cell carcinoma. *Cancer Cell Int* 2021e;**21**:151.
- Wang X, Gu M, Ju Y *et al.* PIK3C3 acts as a tumor suppressor in esophageal squamous cell carcinoma and was regulated by MiR-340-5p. *Med Sci Monit* 2020b;**26**:e920642.
- Wang Y, Zhang J, Zhao W *et al.* MicroRNA expression in esophageal squamous cell carcinoma: novel diagnostic and prognostic biomarkers. *Mol Med Rep* 2017;**15**:3833–3839.
- Wang Z, Cheng Z, Lu S *et al.* Characterization of the intra-tumoral B cell immunoglobulin repertoire is of prognostic value for esophageal squamous cell carcinoma. *Front Immunol* 2022d;**13**:896627.
- Wei M, Zhao L, Lv J *et al.* The mediation effect of serum metabolites on the relationship between long-term smoking exposure and esophageal squamous cell carcinoma. *BMC Cancer* 2021;**21**:415.
- Wen J, Hu Y, Liu Q *et al.* miR-424 coordinates multilayered regulation of cell cycle progression to promote esophageal squamous cell carcinoma cell proliferation. *EBioMedicine* 2018;**37**:110–124.
- Wen J, Luo K, Liu H *et al.* MiRNA expression analysis of pre-treatment biopsies predicts the pathological response of esophageal squamous cell carcinomas to neoadjuvant chemoradiotherapy. *Ann Surg* 2016;**263**:942–948.
- Wen J, Yang H, Liu MZ *et al.* Gene expression analysis of pre-treatment biopsies predicts the pathological response of esophageal squamous cell carcinomas to neo-chemoradiotherapy. *Ann Oncol* 2014;**25**:1769–1774.
- Weng NQ, Chi J, Wen J *et al.* The prognostic value of a seven-lncRNA signature in patients with esophageal squamous cell carcinoma: a lncRNA expression analysis. *J Transl Med* 2020;**18**:47.
- Wolman SR, Heppner GH. Genetic heterogeneity in breast cancer. *J Natl Cancer Inst* 1992;**84**:469–470.
- Wu BL, Xu LY, Du ZP *et al.* MiRNA profile in esophageal squamous cell carcinoma: downregulation of miR-143 and miR-145. *World J Gastroenterol* 2011a;**17**:79–88.
- Wu C, Hu Z, He Z *et al.* Genome-wide association study identifies three new susceptibility loci for esophageal squamous-cell carcinoma in Chinese populations. *Nat Genet* 2011b;**43**:679–684.
- Wu C, Kraft P, Zhai K *et al.* Genome-wide association analyses of esophageal squamous cell carcinoma in Chinese identify multiple susceptibility loci and gene-environment interactions. *Nat Genet* 2012;**44**:1090–1097.
- Wu C, Li D, Jia W *et al.* Genome-wide association study identifies common variants in SLC39A6 associated with length of survival in esophageal squamous-cell carcinoma. *Nat Genet* 2013;**45**:632–638.
- Wu C, Wang C, Guan X *et al.* Diagnostic and prognostic implications of a serum miRNA panel in oesophageal squamous cell carcinoma. *PLoS One* 2014a;**9**:e92292.
- Wu C, Wang Z, Song X *et al.* Joint analysis of three genome-wide association studies of esophageal squamous cell carcinoma in Chinese populations. *Nat Genet* 2014b;**46**:1001–1006.
- Wu Y, Hu L, Liang Y *et al.* Up-regulation of lncRNA CASC9 promotes esophageal squamous cell carcinoma growth by negatively regulating PDCD4 expression through EZH2. *Mol Cancer* 2017;**16**:150.
- Xi Y, Lin Y, Guo W *et al.* Multi-omic characterization of genome-wide abnormal DNA methylation reveals diagnostic and prognostic markers for esophageal

- squamous-cell carcinoma. *Signal Transduct Target Ther* 2022a;**7**:53.
- Xi Y, Shen Y, Wu D et al. CircBCAR3 accelerates esophageal cancer tumorigenesis and metastasis via sponging miR-27a-3p. *Mol Cancer* 2022b;**21**:145.
- Xie Z, Chen G, Zhang X et al. Salivary microRNAs as promising biomarkers for detection of esophageal cancer. *PLoS One* 2013;**8**:e57502.
- Xiong K, Tao Z, Zhang Z et al. Identification and validation of a prognostic immune-related gene signature in esophageal squamous cell carcinoma. *Front Bioeng Biotechnol* 2022;**10**:850669.
- Xu J, Chen Y, Zhang R et al. Global metabolomics reveals potential urinary biomarkers of esophageal squamous cell carcinoma for diagnosis and staging. *Scientific Reports* 2016;**6**:35010.
- Xu J, Chen Y, Zhang R et al. Global and targeted metabolomics of esophageal squamous cell carcinoma discovers potential diagnostic and therapeutic biomarkers. *Mol Cell Proteomics* 2013;**12**:1306–1318.
- Xu J, Li J, Zhang R et al. Development of a metabolic pathway-based pseudo-targeted metabolomics method using liquid chromatography coupled with mass spectrometry. *Talanta* 2019;**192**:160–168.
- Xu P, Wang L, Liu Q et al. The abnormal expression of circ-ARAP2 promotes ESCC progression through regulating miR-761/FOXO1 axis-mediated stemness and the endothelial-mesenchymal transition. *J Transl Med* 2022;**20**:318.
- Xue L, Li W, Fan X et al. Identification of second primary tumors from lung metastases in patients with esophageal squamous cell carcinoma using whole-exome sequencing. *Theranostics* 2020;**10**:10606–10618.
- Xue ST, Zheng B, Cao SQ et al. Long non-coding RNA LINC00680 functions as a ceRNA to promote esophageal squamous cell carcinoma progression through the miR-423-5p/PAK6 axis. *Mol Cancer* 2022;**21**:69.
- Yan T, Cui H, Zhou Y et al. Multi-region sequencing unveils novel actionable targets and spatial heterogeneity in esophageal squamous cell carcinoma. *Nat Commun* 2019;**10**:1670.
- Yan W, Shih J, Rodriguez-Canales J et al. Three-dimensional mRNA measurements reveal minimal regional heterogeneity in esophageal squamous cell carcinoma. *Am J Pathol* 2013;**182**:529–539.
- Yan W, Shih JH, Rodriguez-Canales J et al. Identification of unique expression signatures and therapeutic targets in esophageal squamous cell carcinoma. *BMC Res Notes* 2012;**5**:73.
- Yang F, Wen S, Zhang Y et al. Identifying potential metastasis-related long non-coding RNAs, microRNAs, and message RNAs in the esophageal squamous cell carcinoma. *J Cell Biochem* 2019;**120**:13202–13215.
- Yang H, Su H, Hu N et al. Integrated analysis of genome-wide miRNAs and targeted gene expression in esophageal squamous cell carcinoma (ESCC) and relation to prognosis. *BMC Cancer* 2020a;**20**:388.
- Yang T, Hui R, Nouws J et al. Untargeted metabolomics analysis of esophageal squamous cell cancer progression. *J Transl Med* 2022;**20**:127.
- Yang X, Suo C, Zhang T et al. Targeted proteomics-derived biomarker profile develops a multi-protein classifier in liquid biopsies for early detection of esophageal squamous cell carcinoma from a population-based case-control study. *Biomark Res* 2021;**9**:12.
- Yang Y, Li D, Yang Y et al. An integrated analysis of the effects of microRNA and mRNA on esophageal squamous cell carcinoma. *Mol Med Rep* 2015;**12**:945–952.
- Yang Y-M, Hong P, Xu WW et al. Advances in targeted therapy for esophageal cancer. *Signal Transduct Target Ther* 2020b;**5**:229.
- Yao J, Huang JX, Lin M et al. Microarray expression profile analysis of aberrant long non-coding RNAs in esophageal squamous cell carcinoma. *Int J Oncol* 2016;**48**:2543–2557.
- Yao L, Zhang Y, Zhu Q et al. Downregulation of microRNA-1 in esophageal squamous cell carcinoma correlates with an advanced clinical stage and its overexpression inhibits cell migration and invasion. *Int J Mol Med* 2015;**35**:1033–1041.
- Ye H, Mulmi Shrestha S, Zhu J et al. Long noncoding RNA LINC00491 promotes proliferation and inhibits apoptosis in esophageal squamous cell carcinoma. *Int J Mol Med* 2021a;**47**:33.
- Ye W, Lin Y, Bezabeh T et al. 1 H NMR-based metabolomics of paired esophageal tumor tissues and serum samples identifies specific serum biomarkers for esophageal cancer. *NMR Biomed* 2021b;**34**:e4505.
- Yi J, Jin L, Chen J et al. MiR-375 suppresses invasion and metastasis by direct targeting of SHOX2 in esophageal squamous cell carcinoma. *Acta Biochim Biophys Sin (Shanghai)* 2017;**49**:159–169.
- Yi M, Tan Y, Wang L et al. TP63 links chromatin remodeling and enhancer reprogramming to epidermal differentiation and squamous cell carcinoma development. *Cell Mol Life Sci* 2020;**77**:4325–4346.
- Yokoi A, Matsuzaki J, Yamamoto Y et al. Integrated extracellular microRNA profiling for ovarian cancer screening. *Nat Commun* 2018;**9**:4319.
- You B-H, Yoon J-H, Kang H et al. HERES, a lncRNA that regulates canonical and noncanonical wnt signaling pathways via interaction with EZH2. *Proc Natl Acad Sci U S A* 2019;**116**:24620–24629.
- Yu HX, Wang XL, Zhang LN et al. MicroRNA-384 inhibits the progression of esophageal squamous cell carcinoma through blockade of the LIMK1/cofilin signaling pathway by binding to LIMK1. *Biomed Pharmacother* 2019a;**109**:751–761.
- Yu J, Wu X, Huang K et al. Bioinformatics identification of lncRNA biomarkers associated with the progression of esophageal squamous cell carcinoma. *Mol Med Rep* 2019b;**19**:5309–5320.
- Yu J, Zhu M, Lv M et al. Characterization of a five-microRNA signature as a prognostic biomarker for esophageal squamous cell carcinoma. *Sci Rep* 2019c;**9**:19847.

- Yu M, Wen W, Yi X *et al.* Plasma metabolomics reveals diagnostic biomarkers and risk factors for esophageal squamous cell carcinoma. *Front Oncol* 2022;**12**:829350.
- Yu T, Cao R, Li S *et al.* MiR-130b plays an oncogenic role by repressing PTEN expression in esophageal squamous cell carcinoma cells. *BMC Cancer* 2015;**15**:29.
- Yu Y, Li Z, Huang C *et al.* Integrated analysis of genomic and transcriptomic profiles identified a prognostic immunohistochemistry panel for esophageal squamous cell cancer. *Cancer Med* 2020;**9**:575–585.
- Zang B, Wang W, Wang Y *et al.* Metabolomic characterization reveals ILF2 and ILF3 affected metabolic adaptations in esophageal squamous cell carcinoma. *Front Mol Biosci* 2021a;**8**:721990.
- Zang Q, Sun C, Chu X *et al.* Spatially resolved metabolomics combined with multicellular tumor spheroids to discover cancer tissue relevant metabolic signatures. *Anal Chim Acta* 2021b;**1155**:338342.
- Zhang C, Wang C, Chen X *et al.* Expression profile of microRNAs in serum: a fingerprint for esophageal squamous cell carcinoma. *Clin Chem* 2010;**56**:1871–1879.
- Zhang H, Wang L, Hou Z *et al.* Metabolomic profiling reveals potential biomarkers in esophageal cancer progression using liquid chromatography-mass spectrometry platform. *Biochem Biophys Res Commun* 2017;**491**:119–125.
- Zhang K, Li L, Zhu M *et al.* Comparative analysis of histone H3 and H4 post-translational modifications of esophageal squamous cell carcinoma with different invasive capabilities. *J Proteomics* 2015a;**112**:180–189.
- Zhang L, Zhou Y, Cheng C *et al.* Genomic analyses reveal mutational signatures and frequently altered genes in esophageal squamous cell carcinoma. *Am J Hum Genet* 2015b;**96**:597–611.
- Zhang S, Lu X, Hu C *et al.* Serum metabolomics for biomarker screening of esophageal squamous cell carcinoma and esophageal squamous dysplasia using gas Chromatography-Mass spectrometry. *ACS Omega* 2020a;**5**:26402–26412.
- Zhang X, Peng L, Luo Y *et al.* Dissecting esophageal squamous-cell carcinoma ecosystem by single-cell transcriptomic analysis. *Nat Commun* 2021;**12**:5291.
- Zhang Y, Wang J, Dai N *et al.* Alteration of plasma metabolites associated with chemoradiosensitivity in esophageal squamous cell carcinoma via untargeted metabolomics approach. *BMC Cancer*, 2020b;**20**:835.
- Zhao D, Guo Y, Wei H *et al.* Multi-omics characterization of esophageal squamous cell carcinoma identifies molecular subtypes and therapeutic targets. *JCI Insight* 2024;**9**:e171916.
- Zhao F, Dong Z, Li Y *et al.* Comprehensive analysis of molecular clusters and prognostic signature based on m7G-related lncRNAs in esophageal squamous cell carcinoma. *Front Oncol* 2022a;**12**:893186.
- Zhao F, Li Z, Dong Z *et al.* Exploring the potential of exosome-related lncRNA pairs as predictors for immune microenvironment, survival outcome, and microbiota landscape in esophageal squamous cell carcinoma. *Front Immunol* 2022b;**13**:918154.
- Zhao J, Zhao X, Yu J *et al.* A multi-platform metabolomics reveals possible biomarkers for the early-stage esophageal squamous cell carcinoma. *Anal Chim Acta* 2022c;**1220**:340038.
- Zhao JY, Wang F, Li Y *et al.* Five miRNAs considered as molecular targets for predicting esophageal cancer. *Med Sci Monit* 2015;**21**:3222–3230.
- Zhao X, Wang Y, Meng F *et al.* Risk stratification and validation of eleven autophagy-related lncRNAs for esophageal squamous cell carcinoma. *Front Genet* 2022d;**13**:894990.
- Zhao Y, Wei L, Shao M *et al.* BRCA1-associated protein increases invasiveness of esophageal squamous cell carcinoma. *Gastroenterology* 2017;**153**:1304–1319.e5.
- Zheng D, Ding Y, Ma Q *et al.* Identification of serum microRNAs as novel biomarkers in esophageal squamous cell carcinoma using feature selection algorithms. *Front Oncol* 2018;**8**:674.
- Zheng Y, Chen Z, Han Y *et al.* Immune suppressive landscape in the human esophageal squamous cell carcinoma microenvironment. *Nat Commun* 2020;**11**:6268.
- Zhou J, Wu Z, Wong G *et al.* CDK4/6 or MAPK blockade enhances efficacy of EGFR inhibition in oesophageal squamous cell carcinoma. *Nat Commun* 2017;**8**:13897.
- Zhou PL, Wu Z, Zhang W *et al.* Circular RNA hsa_circ_0000277 sequesters miR-4766-5p to upregulate LAMA1 and promote esophageal carcinoma progression. *Cell Death Dis* 2021;**12**:676.
- Zhu Q, Huang L, Yang Q *et al.* Metabolomic analysis of exosomal-markers in esophageal squamous cell carcinoma. *Nanoscale* 2021a;**13**:16457–16464.
- Zhu T, Ma Z, Wang H *et al.* Immune-related long non-coding RNA signature and clinical nomogram to evaluate survival of patients suffering esophageal squamous cell carcinoma. *Front Cell Dev Biol* 2021b;**9**:641960.
- Zhu Y, Ma Y, Peng H *et al.* MiR-130b promotes the progression of oesophageal squamous cell carcinoma by targeting SASH1. *J Cell Mol Med* 2019a;**23**:93–103.
- Zhu Y, Qi X, Yu C *et al.* Identification of prothymosin alpha (PTMA) as a biomarker for esophageal squamous cell carcinoma (ESCC) by label-free quantitative proteomics and quantitative dot blot (QDB). *Clin Proteomics* 2019b;**16**:12.