SPANDIDOS PUBLICATIONS
ONCOLOGY REPORTS 51: 8, 2024

Copy number variations in esophageal squamous cell carcinoma: Emerging cancer drivers and biomarkers (Review)

JING REN[1], PENGZHOU KONG[2], YANQIANG WANG[2], DAWEI GUO[3] and LING ZHANG[1,2]

[1]School of Basic Medical Sciences; [2]Key Laboratory of Cellular Physiology of The Ministry of Education; [3]School of Forensic Medicine, Shanxi Medical University, Taiyuan, Shanxi 030001, P.R. China

Received January 12, 2023; Accepted September 22, 2023

DOI: 10.3892/or.2023.8667

Abstract. The morbidity and mortality of esophageal squamous cell carcinoma (ESCC) remains high in China. ESCC is significantly influenced by a complex interplay of environmental and genetic factors. Copy number variations (CNVs) are a major form of genome-scale changes in ESCC and are closely related to tumorigenesis and development. Genome-wide detection and analysis allow the identification of important CNV-affected genes with potential clinical applications. In both coding and non-coding regions, CNVs have been identified frequently in certain segments of chromosomes. CNV-impacted genes have crucial roles in multiple cellular processes, including proliferation, apoptosis, metastasis, and metabolic pathways. More importantly, they may serve as potential therapeutic targets for patients with ESCC. Therefore, studying the role of CNVs in ESCC is helpful to explore the pathogenesis of ESCC and to find effective treatment targets, which have profound implications for the diagnosis and therapy of ESCC.

Contents

1. Introduction
2. Mechanisms of formation of CNVs and their effects on gene expression
3. Detection methods for CNVs
4. Affected protein-coding and non-coding CNV genes in ESCC
5. CNVs with clinical application in ESCC
6. Conclusions and future prospectives

Correspondence to: Dr Ling Zhang, School of Basic Medical Sciences, Shanxi Medical University, 56 Xinjian Road, Taiyuan, Shanxi 030001, P.R. China
E-mail: zhanglingty@sxmu.edu.cn

Key words: copy number variations, esophageal squamous cell carcinoma, driver gene, potential target

1. Introduction

Esophageal cancer is a common digestive tract cancer worldwide and ranks seventh in morbidity and sixth in mortality rates among all cancer types. Approximately 604,000 new cases were recorded in 2020 worldwide (1). According to differences in histopathology, esophageal cancer can be divided into two subtypes: Esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). ESCC is the most common type of esophageal cancer worldwide, which is primarily distributed in the Asian esophageal cancer belt, including Japan, northern China, Iran, and parts of Africa (2). With a 5-year survival rate ranging from 15 to 25% in China, there is an urgent need to improve patient survival by improving early diagnosis and treatment (3). Therefore, it is vital to study the pathogenesis of ESCC, to identify prognostic markers and effective drugs for patients.

To date, several genome-wide profiles of ESCC have been performed by whole genome, exome, and targeted sequencing. Compared with EAC, the copy number variations (CNVs) landscape of ESCC is more akin to that of other types of squamous carcinomas, such as head and neck squamous cell carcinoma (HNSCC) and cervical squamous cell carcinoma (CESC) (4-6). In addition to well-established cancer genes (*EGFR*, *CDKN2A/2B*, *CCND1*), researchers have also revealed that other genes (*CBX4*, *ZNF750*, *CDCA7*, *FAM84B*) may play important roles in ESCC (7-10). Moreover, generous focal CNV segments (amplifications and deletions) have been identified. Certain gene signatures in these segments can lead to metastasis or poor prognosis (11). Integrated gene enrichment analysis of somatic mutations and CNVs has found pivotal cancer pathways involved in ESCC progression, including the receptor tyrosine kinase (RTK)-Ras-PI3K, JAK-STAT, Akt, NOTCH, Wnt, cell cycle, and p53 signaling pathways (6,7,12). All these findings highlight potential therapeutic molecular targets for the management of ESCC (13).

Although certain reports have shed light on specific genes related to the molecular carcinogenic mechanism or targeted treatment of ESCC (6,12), a systematic review concerning CNVs has not been conducted yet to the best of our knowledge. The present review aimed to summarize the implications of CNVs on the formation and progression of ESCC; to describe the identified genes that are amplified or deleted in ESCC; and to discuss their roles as potential biomarkers. A summary of

the present review is presented in Table I. The current review may help to understand the genomic characteristics and variations in ESCC, thus providing a guideline for targeted therapy.

2. Mechanisms of formation of CNVs and their effects on gene expression

CNVs are widely distributed across the human genome and refer to the copy number change by deletion, insertion, inversion, translocation, duplication, and other types of chromosomal rearrangements (14). CNVs are generally DNA segments of >1 kb in size, with a mean fragment length of ~2.9 kb (15,16). CNVs are mainly generated by non-allele homologous recombination (NAHR), non-homologous end joining (NHEJ), a DNA replication-based mechanism such as fork stalling and template switching (FoSTeS), and long interspersed nuclear element-1 (L1) retrotransposition (17). NAHR is the principal mechanism for the formation of CNVs (18). DNA is damaged and repaired via a DNA double-stranded break repair mechanism to produce NHEJ following exposure to ionizing radiation or oxidative stress (19,20). In 2007, Lee *et al* (21) proposed a FoSTeS model to explain the complex CNV rearrangements, which may explain the process of producing more complex and longer CNVs. Retrotransposon is primarily produced by spontaneous transposon L1s (22).

CNVs play a prominent role in tumorigenesis in various cancer types, including breast cancer, ovarian cancer, hepatocellular carcinoma, colorectal cancer, and bladder carcinoma (23-27). Amplified genomic regions are often detected to harbor oncogenes, while tumor suppressor genes are often located in the deleted regions. The molecular mechanisms of pathogenesis primarily include gene dosage, gene disruption and fusion, position effect, and recessive allele mutations (18). The gene dosage effect is characterized by changes in gene expression levels along with the CNVs. Gene breakups and breakpoint fusions are caused by interruptions directly by CNVs, resulting in loss of function or gain of function mutations. The position effect leading to rearrangement regulates distal and proximal gene expression, and affects the translation of adjacent genes (28,29).

3. Detection methods for CNVs

In recent years, with the rapid development of sequencing technologies, the methods of detecting CNVs have made notable progress. Common detection methods for CNVs can be primarily divided into two categories: i) Genome-wide detection of unknown CNVs and ii) specific detection of known CNVs. Microarray and sequencing are commonly used to detect genome-unknown CNVs.

Currently, the mainstream approaches for analyzing CNVs are gene sequencing-based methods. Sequencing techniques comprise Sanger DNA sequencing, next-generation sequencing (NGS), and third-generation sequencing (TGS), of which NGS and TGS are commonly used. Whole genome sequencing (WGS), whole exome sequencing (WES), and targeted sequencing (TS) are frequently utilized for CNV detection by NGS or TGS. Each kind of sequencing may be applied to different aspects. WGS is a high-resolution and reliable approach to detect CNVs with greater coverage and increased accuracy (30,31). Compared to WGS, WES needs less time and is cheaper, but its application is limited. Since exomes account for 1-1.5% of the human genome, WES is a better choice when only detecting coding region copy number changes. TS focuses on select DNA segments that can be used to identify somatic changes (32). In recent decades, NGS has revealed aberrant genes and signaling pathways, and has certain advantages regarding throughput capacity and price. TGS can be used to screen out single molecular genes even without PCR sequencing, and can generate longer and more accurate reads with faster speed (33,34). It can read >15 kb on average, which provides the possibility of obtaining longer sequences and avoids the need for fragment rejoining (35). By TGS, CNV fragment length, and specific sites can be detected more precisely. However, it has some drawbacks such as low throughput, and high cost and error rate.

Chromosome microarrays primarily consist of comparative genomic hybridization and single nucleotide polymorphisms. Candidate-gene screening is generally utilized to identify the CNVs of specific sites. High-throughput gene sequencing, chromosome microarray, and candidate-gene screening can be used for further detection (Fig. 1).

4. Affected protein-coding and non-coding CNV genes in ESCC

CNVs are involved in the initiation and development of ESCC (5). CNVs at the chromosomal level were present in 97.9% of patients with ESCC (4). Previous studies, some of them conducted by our group, have identified frequent CNV fragments, including gains at 3q, 5p, 7p, 7q, 8p, 8q, 12p, 14q, 16p, 17p, 18p, 20p, 20q, Xp, and Xq, and losses at 3p, 4p, 4q, 5q, 9p, 10p, 11p, 13q, 18q, 19p, 19q, and 21q (4-8). Certain genes have been identified with copy number changes, including *CCND1*, *CTTN*, *MYEOV*, *EGFR*, *FGFR1*, *ZNF750*, *SOX2*, and *FBXW7*, in ESCC (7,10,36). There are several studies focused on the screening of non-coding RNA, including microRNA (miR)-21, miR-23a, miR-342-3p, miR-483-5p, miR-548d-5p, miR-574-5p, miR-595, miR-766, miR-940, miR-1224-3p, miR-1260, miR-1280, miR-4448, and miR-4707-5p, which are overexpressed with amplifications, while let-7c, miR-139-3p and miR-139-5p are downregulated with losses (37,38). Due to genomic and epigenomic aberrations, several signaling pathways such as disrupted G_1-S cell cycle and amplified PI3K/Akt/mTOR could be therapeutic targets for further treatment (36,39,40). Our previous study constructed an evolutionary tree of ESCC and found three models of evolution: i) Metastasis-to-metastasis; ii) linear spread; and iii) explosive metastasis models. The majority of amplifications or deletions of driving genes that play critical roles in tumor pathogenesis are trunk variations. Furthermore, cell cycle-regulated genes occur in the early stages of tumor progression in particular (12). Our group found that some local deletions were closely associated with a poor prognosis, including 13q12.11, 13q14.2, 17q25.3, and 22q13.33 (10). As mentioned above, CNVs can be clustered according to different locations at a chromosomal arm level. The following sections summarize important CNVs identified in ESCC (Fig. 2 and Table II).

Table I. Highlights of the article.

Highlights	(Refs.)
The complex formation mechanisms of CNVs including NAHR, NHEJ, FoSTeS, retrotransposon, amongst others.	(17-22)
The diversity of detection methods for CNVs including WGS, WES, TS, chromosome microarrays, amongst others.	(30-35)
The universality of CNVs affected genes including *NFE2L2*, *ZNF750*, amongst others.	(41-99)
The potential value of clinical application of CNV events including cetuximab and nimotuzumab in EGFR, Apatinib and Endor in VEGFR, amongst others.	(100-110)

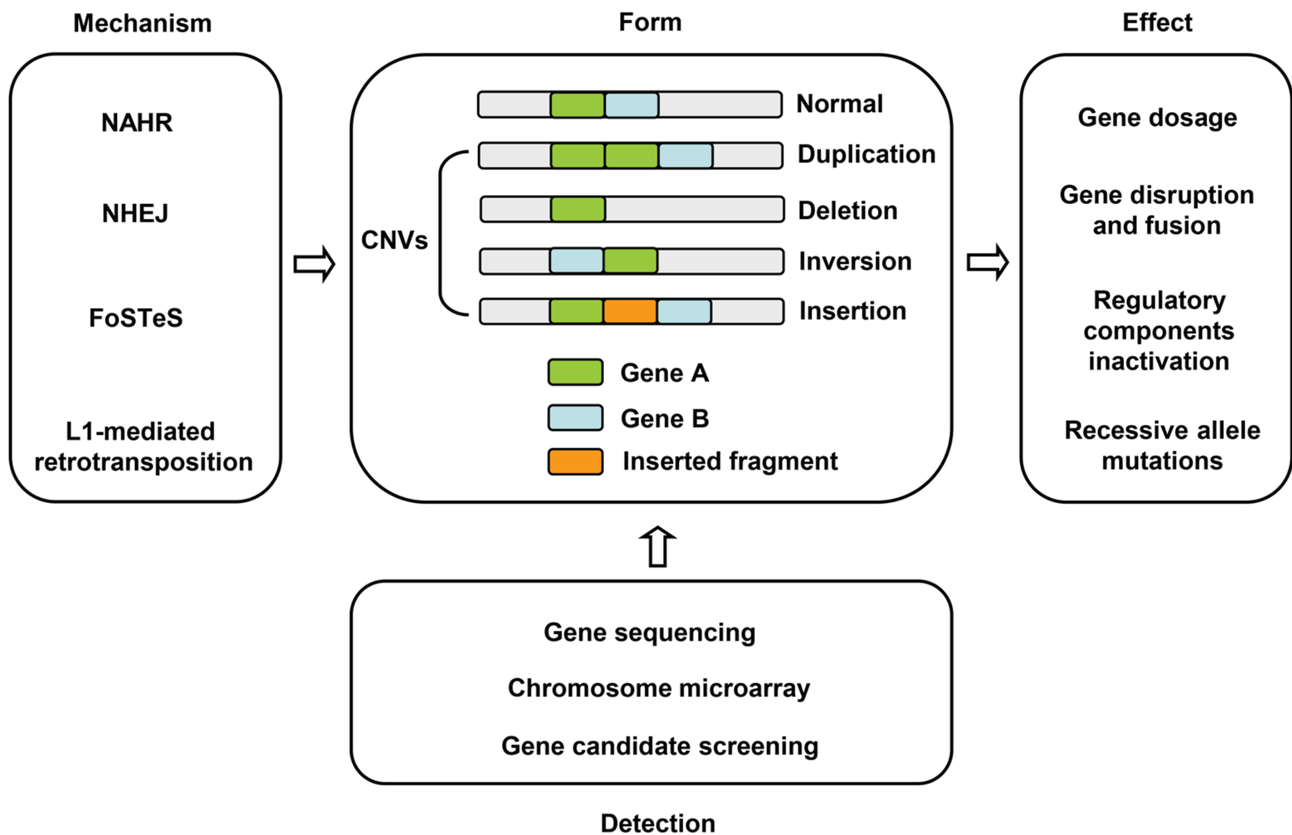

Figure 1. Diagram of the mechanisms, forms, effects, and detection methods of copy number variations.

Chromosome 1

1p36.32. Previous studies on 1p36.32 have demonstrated that CNVs in long non-coding RNA (lncRNA) regions play critical roles in cancer progression. Importantly, certain lncRNAs may serve as prognostic biomarkers for clinical diagnosis. For example, lncRNA *TP73-AS1* is located in 1p36.32 and is upregulated in ESCC, promoting proliferation and suppressing apoptosis. *TP73-AS1* enhances the sensitivity of ESCC to cisplatin and 5-fluorouracil. In clinical samples, *TP73-AS1* is associated with TNM staging and tumor localization of ESCC, but not with sex, age, lymphatic metastasis, or differentiation. Increased copy numbers of *TP73-AS1* were found in early-stage ESCC, whereas decreased expression of *TP73-AS1* was found in advanced TNM stages (41).

1q32.1. Situated on chromosome 1q32.1, *AGPG* is identified as a metabolic lncRNA. According to Liu *et al* (42), the copy number of *AGPG* in ESCC cell lines was increased compared with normal cells. As a transcriptional target for p53, *AGPG* plays a crucial role in promoting glycolysis and proliferation via PFKFB3, eventually leading to carcinogenesis. Furthermore, overall survival (OS) analysis revealed that advanced *AGPG* expression was indicative of a poor prognosis for ESCC, showing that it may be a promising target for metabolism-related treatment for ESCC.

Chromosome 2

2q31.2. Large-scale chromosome amplification of the *NFE2L2* gene was commonly found in ESCC samples (43). *NFE2L2* was not only amplified in ESCC but also mutated in patients (10). NFE2L2 (also known as NRF2) is a transcription factor strongly activated in ESCC. Elevated expression of NRF2 is indicative of a poor outcome, and has been found to be related to metabolism, as well as Notch and PI3K/Akt signaling (44). As an independent prognostic indicator in ESCC, upregulation of the *NRF2* gene is important for lymph node metastasis, postoperative recurrence, and OS (45-47). Inhibitors such as

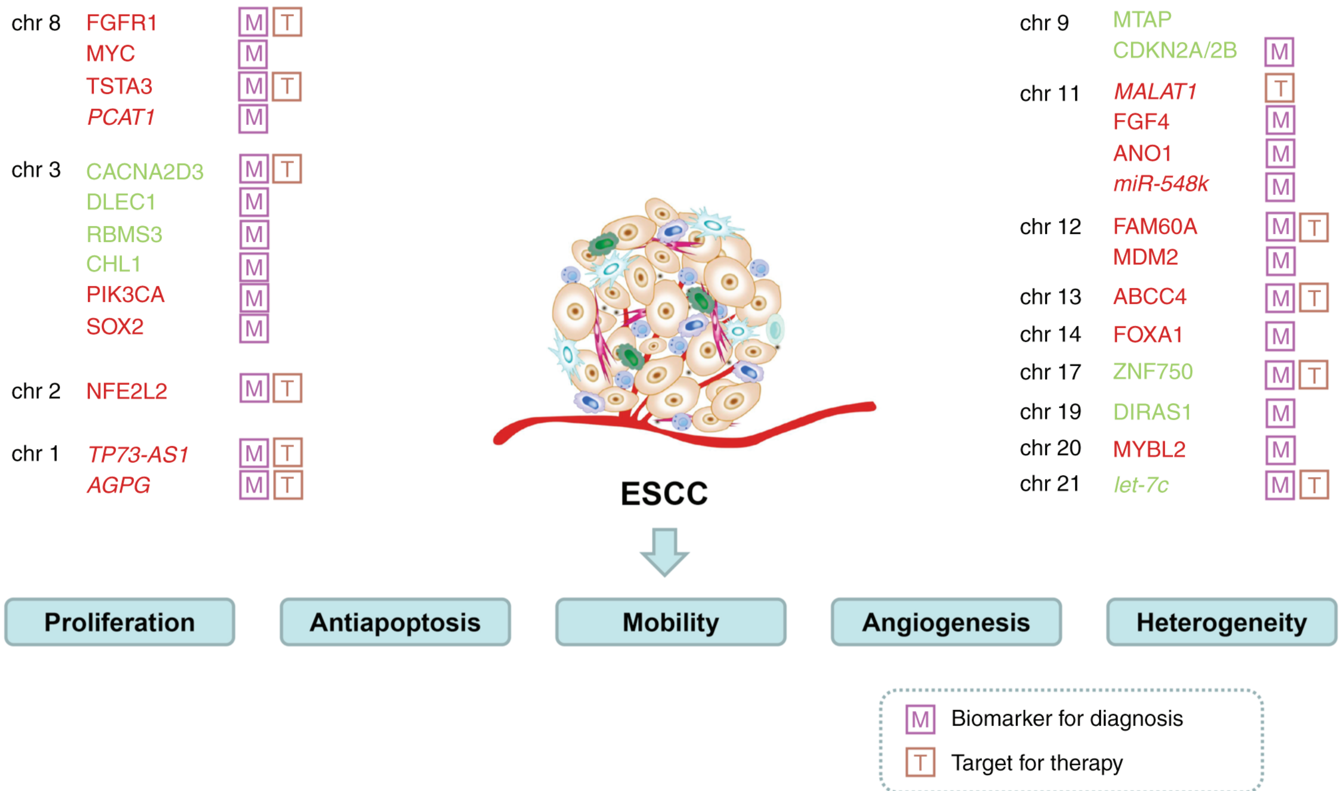

Figure 2. Overview of copy number variation-affected genes during the progression of esophageal squamous cell carcinoma. These genes have a close association with the characteristics of cancer, such as proliferation, anti-apoptosis, mobility, angiogenesis, and heterogeneity. Genes colored red indicate amplification or gain, while those colored green indicate deletion or loss. M, biomarker for diagnosis; T, therapeutic target.

AEM1 and ML385 can block signaling pathways and attack tumors (48,49). NRF2 is associated with chemoradiation; increased expression of NRF2 in the nucleus is predictive of a poor clinical response and shorter progression-free survival after chemoradiotherapy in patients with ESCC.

Chromosome 3

3p21-3p26. *CACNA2D3*, which is located at 3p21.1, is a tumor suppressor gene. Reduced expression of CACNA2D3 is significantly associated with lymph node metastasis, TNM staging, and a poorer prognosis in patients with ESCC. A functional study showed that CACNA2D3 inhibited tumorigenesis and cell migration, and induced apoptosis (50). Moreover, CACNA2D3 enhanced the sensitivity of ESCC to cisplatin by inducing calcium-mediated apoptosis and inhibiting the PI3K/Akt signaling pathway. Thus, regulating CACNA2D3 expression is a potential novel strategy for improving the effectiveness of cisplatin in patients with ESCC (51). *DLEC1*, which is located at 3p22.2, is frequently downregulated not only in lung and nasopharyngeal carcinoma cell lines, but also in ESCC and other primary tumors. DLEC1 is continuously expressed in normal tissues and immortalized epithelial cells. However, mutations in the *DLEC1* gene have rarely been detected. Promoter CpG methylation and histone acetylation leads to low expression of DLEC1. Promoter CpG methylation of *DLEC1* is frequently detected in ESCC, and is closely associated with lymph node metastasis, tumor recurrence, and progression. DLEC1 inhibits cell proliferation via cell cycle arrest and metastasis. In addition, it can inhibit cell metastasis by reversing epithelial-mesenchymal transformation (EMT) and stemness via the STAT3 signaling pathway (52). Decreased expression of *RBMS3* in 3p24 leads to a poorer prognosis in ESCC. Genetic loss of RBMS3 can effectively reduce the oncogenicity of ESCC tumor cells *in vivo* and *in vitro*. It has been demonstrated that RBMS3 dysregulates the expression of MYC and CDK4, thereby inhibiting the phosphorylation of Rb (53). *CHL1*, which is located at 3p26, is another important tumor suppressor gene. The deletion of CHL1 is associated with poor differentiation, invasion, lymph node metastasis, tumor progression, and a worse OS rate in ESCC. The anti-proliferative and metastatic factor CHL1 can inhibit cell adhesion via the AKT signaling pathway (54).

3q26. The 3q26 segment has been found to be amplified in various tumors such as cervical, ovarian, lung, and head and neck cancer (55). The 3q26 region is notably highly amplified in ESCC, and harbors oncogenes such as *PIK3CA*, *PRKCI*, *MDS1-EVI1*, and *SOX2* (56-58). As an oncogene, *PIK3CA* encodes the catalytic subunit p110-kinase of PI3K, which is highly expressed in esophageal cancer and is involved in the PI3K signaling pathway to promote the development of ESCC (57,59). Gene amplification results in overexpression of PRKCI. Immunohistochemical analysis using 180 ESCC samples showed that overexpression of PRKCI was correlated with lymph node metastasis, while upregulated expression of PRKCI was correlated with poor prognosis in ESCC (56). SRY-box transcription factor 2 (SOX2) has been evaluated in several studies. For instance, Gen *et al* (60,61) reported the role of *SOX2* as a protooncogene in ESCC. The change in

Table II. Copy number variations associated with esophageal squamous cell carcinoma.

Chromosome location	Associated genes	Variation type	Frequency (%)	Function	Mechanisms	Clinical implications	(Refs.)
1p36.32	*TP73-AS1*	Gain	2.1	Oncogene	Promote proliferation, suppress apoptosis, and increase the chemical sensitivity to cisplatin and 5-FU	An independent prognostic biomarker and a potential therapeutic target	(41)
1q32.1	*AGPG*	Gain	1.1	Oncogene	Promote glycolysis and proliferation	A promising target for metabolism-related treatment	(42)
2q31.2	*NFE2L2*	Gain	14.3	Oncogene	Associate with metabolic pathways, Notch signaling pathway and PI3K/Akt signaling pathway	A predictive marker used in chemoradiotherapy	(44-49)
3p21.1	*CACNA2D3*	Loss	9	Suppressor gene	Inhibit tumorigenesis, mobility, induce apoptosis and enhance the chemical sensitivity to cisplatin	A potential target when combined with cisplatin	(50,51)
3p22.2	*DLEC1*	Loss	40.2	Suppressor gene	Inhibit cell cycle and metastasis, inhibit JAK/STAT3, MAPK/ERK, Wnt/β-catenin and AKT pathways	DLEC1 methylation as a biomarker in ESCC	(52)
	RBMS3	Loss	61	Suppressor gene	Reduce the tumorigenicity of ESCC tumor cells	A novel therapeutic target	(53)
3p26	*CHL1*	Loss	41	Suppressor gene	Anti-proliferation, anti-metastasis. Inhibit AKT signaling pathway involved in cell adhesion	Unknown	(54)
3q26	*PIK3CA*	Gain	29	Oncogene	Involve in PI3K signaling pathway to promote ESCC	A predictive marker	(57,59)
	SOX2	Gain	28	Oncogene	Activate HIF-1α/STAT3 signaling pathway and promote metastasis	Indicative of a poor prognosis	(60-63)
8p11	*FGFR1*	Gain	9	Oncogene	Activate MEK-ERK signaling pathway	A potential therapeutic target	(13,36,64-67)
8q24	*MYC*	Gain	43	Oncogene	Involve in TLR7/NF-κB, PI3K/AKT and other signaling pathways	An independent prognostic factor	(11,68-71)
	TSTA3	Gain	13	Oncogene	Promote metastasis by regulating fucosylation of LAMP2 and ERBB2	An independent indicator of poor prognosis	(72,73)
	PCAT1	Gain	18	Oncogene	Sponge miR-326 as a ceRNA	A non-invasive biomarker	(38,114)
9p21.3	*MTAP*	Loss	28	Suppressor gene	Induce EMT through the GSK3β/Slug/E-cadherin signal axis	Unknown	(74)
	CDKN2A/2B	Loss	63/56	Suppressor gene	Deregulate G1 phase during the cell cycle	A marker of sensitivity to CDK4/6 inhibitors	(8,75-77)
11q13	*MALAT1*	Gain	22.2	Oncogene	Inhibit the effect of radiotherapy	A potential target in radiotherapy	(115,116)
	FGF4	Gain	54	Oncogene	Unknown	An independent prognostic factor	(80)

Table II. Continued.

Chromosome location	Associated genes	Variation type	Frequency (%)	Function	Mechanisms	Clinical implications	(Refs.)
	ANO1	Gain	57	Oncogene	Promote proliferation, invasion and migration	A predictive marker	(37,81,82)
	mir-548k	Gain	57	Oncogene	Facilitate lymphatic metastasis and regulate microenvironment through the secretion of VEGFC	A predictive marker in early diagnosis	(6,83)
12q11.21	*FAM60A*	Gain	9	Oncogene	Regulates cell cycle, promote cell proliferation, and inhibit apoptosis	A therapeutic target	(84)
12q15	*MDM2*	Gain	5	Oncogene	Promote cell proliferation and inhibit apoptosis	A predictive marker used in radiochemotherapy	(85-88)
13q32.1	*ABCC4*	Gain	14.8	Oncogene	Promote the proliferation and activate oncogenic pathways	A predictive marker and therapeutic target	(89)
14q21.1	*FOXA1*	Gain	8.3	Oncogene	Promote invasion and migration	A predictive marker	(90-92)
17q25.3	*ZNF750*	Loss	14	Suppressor gene	Inhibit tumor proliferation, invasion, migration and angiogenesis	A novel therapeutic target	(93,94)
19p13.3	*DIRAS1*	Loss	59.3	Suppressor gene	Promote cell apoptosis and inhibit invasion	A predictive marker	(98)
20q13.12	*MYBL2*	Gain	51.4	Oncogene	Induce ESCC cell proliferation and regulate cell cycle	An independent indicator of poor prognosis	(38,99)

copy number of SOX2 was positively correlated with SOX2 expression levels (62). SOX2 promotes ESCC and leads to a poorer prognosis in ESCC. High expression of SOX2 is related to differentiation in ESCC (60). By regulating Slug, SOX2 activates the HIF-1α/STAT3 signaling pathway and promotes metastasis in ESCC (63). These aforementioned studies suggest that 3q26 is a vital region for the tumorigenesis of ESCC.

Chromosome 8

8p11. The *FGFR1* gene, which is situated in 8p11, is significantly amplified in ESCC (36). Patients with high FGFR1 expression typically have a short disease-free survival. FGFR1 is an independent predictor of prognosis (64). In other solid tumors, FGFR1 amplification can be used as a hallmark for the prediction of the effectiveness of targeted inhibitors and therefore may similarly act as a promising therapeutic target in ESCC (36). By analyzing >500 cancer cell lines, it was suggested that gains of FGFR1 may be the most effective marker for determining the sensitivity of FGFR1 inhibitors (65). Previous *in vitro* experiments demonstrated that PD173074 prevented cell viability and blocked the MEK-ERK signaling pathway. Anti-FGFR1 drugs such as PD173074 may be used to inhibit ESCC growth, which may be applied in targeted therapies in the future (64,66). The FGFR1 inhibitor AZD4547 can enhance the sensitivity of ESCC cells to gefitinib (67). The results of multiple clinical trials indicated that the small-molecular inhibitor of FGFR1, dovitinib, exhibited good efficacy in ESCC (13).

8q24. The 8q24 chromosomal region contains the *MYC* gene. Amplification of the *MYC* gene is indicative of a poorer prognosis and is an independent prognostic factor. In the majority of clinical cases of ESCC, MYC was closely associated with clinical stage, degree of histological differentiation, lymph node metastasis, and invasive depth of ESCC (11,68). MYC can affect the development of ESCC through multiple signaling pathways such as the TLR7/NF-κB and PI3K/AKT signaling pathways (69-71). Furthermore, GDP-L-fucose synthase (TSTA3) was focally amplified in this region and was significantly associated with poor outcomes of patients with ESCC according to our previous study (72). TSTA3 may act as a driver gene during metastasis by regulating the fucosylation of LAMP2 and ERBB2. Thus, fucosylation inhibitors may be applied to suppress ESCC metastasis (72,73).

Chromosome 9

9p21.3. 9p21.3 is the most frequently deleted region in ESCC. In 9p21.3, the *MTAP* gene is adjacent to *CDKN2A/2B*, which is a tumor suppressor gene. *MTAP* induces EMT through the GSK3β/Slug/E-cadherin signaling axis to promote ESCC metastasis (74). The loss of *CDKN2A/2B* occurs frequently, and is closely associated with lymph node metastasis and poor prognosis in ESCC (8,75). Deficiency of *CDKN2A/2B* is a marker of sensitivity to CDK4/6 inhibitors, which deregulates the G_1 phase of the cell cycle in ESCC (76,77). The 9p region often shows a loss of heterozygosity.

Chromosome 11

11q13.2-11q13.4. The 11q13.2-11q13.4 region is frequently amplified in ESCC and other various tumors, such as HNSCC and breast cancer (78,79). Amplification of 11q13.3 indicates poor outcomes in patients with ESCC (10). The 11q13.2-11q13.4 segment contains 'FGFs-FADD-SHANK2'. Moreover, the *MYEOV*, *CCND1*, *CPT1A*, *CTTN*, *PPFIA*, *FADD*, *TMEM16*, and *CTTS* genes are also located in this region. FGFs include FGF3, FGF4, and FGF19. Upregulated expression of SHANK2 contributes to a poor prognosis of ESCC (38). *FGF4* gene amplification is associated with clinical stages and can be used to predict 5-year survival and recurrence rates. Serving as an independent prognostic factor, FGF4 upregulation is indicative of a shorter OS and disease-free survival (80). The *ANO1* gene, also known as *TMEM16A* or *DOG1*, encoding the Ca^{2+}-activated Cl^- channel protein, is an oncogene that can facilitate cell proliferation, invasion, and migration. Upregulated ANO1 expression is associated with a poor prognosis, and is significantly associated with lymphatic metastasis and advanced TNM stages. Immunohistochemical experiments demonstrated that ANO1 was upregulated in tumor specimens from patients with ESCC (37). Previous *in vivo* and *in vitro* studies demonstrated that the downregulation of ANO1 via the TGF-β signaling pathway could significantly inhibit the carcinogenesis of ESCC (81). ANO1 may thus be used as a biomarker for ESCC (82).

miR-548k has been identified as an oncogene that is present in the 11q13.3-13.4 amplicon, which induces malignant phenotypes of ESCC (6). In addition, miR-548k notably facilitated lymphatic metastasis and regulated the microenvironment through increased secretion of VEGFC. miR-548k and VEGFC may thus have potential as biomarkers for the early diagnosis of their clinical features in serum detection, and may thus serve as novel targets for the prognostic prediction of ESCC (83).

Chromosome 12

12q11.21. FAM60A is also known as SIN3-HDAC Complex Associated Factor. Gains of copy numbers lead to the upregulation of FAM60A in ESCC. Thus, as a driver gene of ESCC, it may be a clinical target. FAM60A is upregulated in tumor tissues of ESCC, and is closely associated with tumor size, lymph node metastasis, and TNM staging. Knockdown of the *FAM60A* gene can repress FAM60A expression, arrest cell cycle events at the G_2/M phase, reduce the proportion of G_1 phase cells, inhibit cell proliferation, and promote apoptosis. FAM60A contributes to metastasis of ESCC, and is predictive of a poor outcome (84).

12q15. *MDM2* is located on 12q15, and exhibits gains in 9% of ESCC cases. In a previous study, *MDM2* gene amplification notably influenced overall prognosis and led to a shorter survival time (85). MDM2 could promote cell proliferation and apoptosis resistance (86). MDM2 negatively regulates p53 protein activity and plays a vital role in p53-mediated apoptosis, aging, and cell cycle arrest. Nutlin-3, an antagonist of MDM2, stabilizes p53 and increases p53 expression in wild-type p53 cells. Radiotherapy combined with Nutlin-3 can notably inhibit wild-type p53 cells and enhance sensitivity to radiotherapy (87). MDM2 can also be used as a predictor of chemoresistance in ESCC phase III (88).

Chromosome 13

13q32.1. Gains of the *ABCC4* gene were found in ESCC. Decreased expression of ABCC4 can result in reduced activity

of COX-2, the PGE2 receptor, and MYC, therefore inhibiting the activity and proliferation of ESCC cells. High expression of ABCC4 indicates poor prognosis. ABCC4 activates oncogenic pathways, thereby promoting carcinogenesis in ESCC. ABCC4 may thus have predictive and therapeutic value for the management of ESCC (89).

Chromosome 14

14q21.1. The *FOXA1* gene, also known as *HNF3A*, is located in a frequently amplified region and functions as a DNA-binding transcription factor (90). FOXA1 expression is correlated with differentiation, vascular invasion, lymphatic metastasis, and subtyping (91). Knockdown of the *FOXA1* gene markedly reduced invasion and migration in ESCC (91). Accordingly, FOXA1 is predictive of a poor prognosis in patients with ESCC (92).

Chromosome 17

17q25.3. The *ZNF750* gene is focally deleted in ESCC (93). Decreased *ZNF750* gene expression promotes tumor proliferation, invasion, migration, and angiogenesis (93,94). ZNF750 directly binds to the promoter of SNAI1 to suppress EMT in ESCC (94). Deletions of ZNF750 indicate advanced malignancy and a poor clinical outcome, which highlights its potential as a therapeutic target for the management of ESCC.

Chromosome 19

19p13.3. Deletion of 19p is quite common in lung cancer, ovarian cancer, and ESCC (95-97). The Ras-like small GTPase *DIRAS1* at 19p13.3 is a tumor suppressor gene. DNA copy number loss leads to downregulation of DIRAS1, while reduced expression of DIRAS1 contributes to metastasis, and poor outcomes in patients with ESCC. DIRAS1 can promote cell apoptosis via dephosphorylating Bad via the ERK1/2 and p38 MAPK signaling pathways, while inhibiting invasion by regulating MMP2 and MMP9 (98).

Chromosome 20

20q13.12. With tandem amplified somatic CNVs, the *MYBL2* gene is upregulated in tumor tissue with copy number gains, which indicates a poorer prognosis (38,99). As a nuclear protein, MYBL2 overexpression can induce cell proliferation, as well as regulate the cell cycle during the S and G_2/M phases in ESCC (99). Deletion of MYBL2 reduced the levels of the cell cycle-related proteins CDK1 and cyclin B1 in ESCC cells. Knockdown of MYBL2 also inhibited ESCC cell proliferation and migration (38).

5. CNVs with clinical application in ESCC

Research on CNVs aims to identify candidate genes and provide clues for the development of effective ESCC clinical treatment. To date, copy number variant genes that have been used in clinical targeted therapy of ESCC include EGFR and VEGFR. Mapped on chromosome 7p11.2, amplified EGFR belongs to the ErbB family of RTKs, and can transfer the downstream signal to the RAS-RAF-MEK-ERK-MAPK and PI3K-AKT-mTOR pathways (100). With an amplification rate of 24.3%, excessive activation of EGFR leads to cell proliferation, invasion, metastasis and poor prognosis in ESCC (6,101). As a well-known valid target for various tumors, EGFR is considered to have potential in ESCC treatment. Thus far, several drugs have been applied to block EGFR signaling such as the monoclonal antibodies cetuximab and nimotuzumab, and the small molecular inhibitor gefitinib. Combination of cetuximab with other adjuvant therapies such as chemotherapeutic drugs and radiotherapy has been shown to exert a remarkable effect on reducing ESCC progression (102). However, the adverse effects need to be taken into consideration. Nimotuzumab exhibits low toxicity, and may thus be a promising treatment strategy for advanced or metastatic ESCC (103). In addition to EGFR, VEGFR also performs well as an effective therapeutic target. VEGFR binds to VEGF to form the VEGF/VEGFR complex and trigger the PI3K/AKT and MAPK/ERK signaling pathways (104). Apatinib and Endor, two anticarcinogens independently developed by Chinese scientists that target the VEGF/VEGFR axis, have been evaluated in clinical trials and may become novel drugs for patients with ESCC (105-107).

Another target, HER-2, has been used for various tumors, particularly breast cancer (108). HER-2 may have potential for the treatment of esophageal cancer as well. In a clinical phase III trial, patients with advanced esophageal adenocarcinoma received combined treatment of the HER-2 specific inhibitor lapatinib and CapeOx; OS reached 12.2 months compared with that of the untreated group (10.5 months), showing a distinct curative effect in esophageal adenocarcinoma (109). At present, clinical evaluation of lapatinib is still required in ESCC. In addition to the utilization of copy number variant genes for targeted therapy, they may also be used for chemoradiotherapy. The MYC locus is highly amplified with a rate of 43% and is expressed throughout the entire process of chemoradiotherapy, which may facilitate an increase in resistance (4). Furthermore, a focal copy-number increase in MYC is markedly associated with a reduced survival rate (110). With an increased understanding of ESCC, additional targets are expected to be identified in the future.

6. Conclusions and future prospectives

CNVs play crucial roles in ESCC occurrence and development by altering numerous protein-coding genes. Future studies should focus on identifying the emerging functions of non-coding RNAs [including miRNAs, lncRNAs, and circular RNAs (circRNAs)] in copy number aberrations, since protein-coding sequences constitute only 2% of the total genome sequence, while the remaining 98% corresponds to non-coding sequences. However, the function of non-coding RNA has not yet been fully elucidated with regard to CNVs.

The expression of lncRNAs is more specific, particularly in cells, tissues, and during the developmental stages, and in diseased tissues (111,112). Compared with linear RNA, circRNAs are more stable, abundant, and better conserved. Thus, they may serve as a potential target. For instance, lower expression of deleted miRNA *let-7c* is correlated with a poorer prognosis and reaction to chemotherapy, and it may be used to predict clinical effects. *In vitro, let-7c* suppresses the IL6/STAT3 signaling pathway after cisplatin exposure. In short, *let-7c* is a therapeutic target for ESCC and is deserved of further research and exploitation for the management of cancer (37,113). Amplified lncRNA *PCAT1* acts as an

oncogene and sponges miR-326 as a competing endogenous RNA. *PCAT1* was notably detected in exosomes secreted by ESCC cells, highlighting its potential as a non-invasive biomarker for patients with cancer (38,114). Overexpression of the lncRNA *MALAT1* may result from frequent amplification of 11q13.1 in ESCC (115). *MALAT1* inhibits the effect of radiotherapy partly due to enhanced Cks1 expression. These results demonstrate that MALAT1-targeted therapy may be feasible for improving the effectiveness of radiotherapy (116). Though promising, non-coding RNA-based therapies remain in an early stage of development and need further validation in confirmatory clinical trials. Numerous studies have been conducted to investigate ESCC; however, ESCC still lacks efficient marker genes for diagnosis and subtyping, unlike *HER2* in breast cancer, and *EGFR* and *ROS* in lung cancer.

Tumors are comprised of not only cancer cells but also various stromal cells, which form the microenvironment the tumor is situated in, and this contributes to cell proliferation, drug resistance, invasion, migration, and the poor outcomes of clinical treatment (117,118). In addition, intra-tumor heterogeneity may lead to differences in the therapeutic efficacy when treating malignant tumors (119). However, the heterogeneity and tumor components of ESCC have not been clearly elucidated yet. Although admixed samples of diverse cell types have been analyzed by second-generation or TGS, heterogeneity was not noticeably detected (120). Single-cell sequencing offers the possibility of exploring genuine cellular and molecular components during tumorigenesis (121). Tumor cells showed higher CNV levels compared with normal epidermal cells in ESCC analyzed at the single-cell level, which exhibited remarkable heterogeneity (122). However, further research is needed to explore the microenvironment of ESCC and to identify more effective targets for tumor therapy.

In conclusion, this review comprehensively summarized the CNV events and CNV-affected genes in ESCC, which contributed to a deeper understanding of the molecular mechanisms underlying the occurrence, development, recurrence, and metastasis of ESCC. Improving our understanding of CNVs in ESCC will be valuable for identifying biomarkers for early diagnosis and molecular typing, as well as prognostic markers, and novel therapeutic targets for the management of ESCC.

Acknowledgements

Not applicable.

Funding

This study was supported by the National Natural Science Foundation of China (grant no. 81972613 and 82072746), the Fundamental Research Program of Shanxi Province (grant no. 202203021222253), the Science and Technology Innovation Team of Shanxi Province (grant no. 202204051001024) and the Science and Technology Achievements Transformation Project of Shanxi Province (grant no. 202204021301062).

Availability of data and materials

Not applicable.

Authors' contributions

JR prepared the manuscript. LZ conceived the subject of review. PK, YW, and DG reviewed and edited the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Patient consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Smyth EC, Lagergren J, Fitzgerald RC, Lordick F, Shah MA, Lagergren P and Cunningham D: Oesophageal cancer. Nat Rev Dis Primers 3: 17048, 2017.
3. Pennathur A, Gibson MK, Jobe BA and Luketich JD: Oesophageal carcinoma. Lancet 381: 400-412, 2013.
4. Chang J, Tan W, Ling Z, Xi R, Shao M, Chen M, Luo Y, Zhao Y, Liu Y, Huang X, *et al*: Genomic analysis of oesophageal squamous-cell carcinoma identifies alcohol drinking-related mutation signature and genomic alterations. Nat Commun 8: 15290, 2017.
5. Chen XX, Zhong Q, Liu Y, Yan SM, Chen ZH, Jin SZ, Xia TL, Li RY, Zhou AJ, Su Z, *et al*: Genomic comparison of esophageal squamous cell carcinoma and its precursor lesions by multi-region whole-exome sequencing. Nat Commun 8: 524, 2017.
6. Song Y, Li L, Ou Y, Gao Z, Li E, Li X, Zhang W, Wang J, Xu L, Zhou Y, *et al*: Identification of genomic alterations in oesophageal squamous cell cancer. Nature 509: 91-95, 2014.
7. Zhang L, Zhou Y, Cheng C, Cui H, Cheng L, Kong P, Wang J, Li Y, Chen W, Song B, *et al*: Genomic analyses reveal mutational signatures and frequently altered genes in esophageal squamous cell carcinoma. Am J Hum Genet 96: 597-611, 2015.
8. Cheng C, Zhou Y, Li H, Xiong T, Li S, Bi Y, Kong P, Wang F, Cui H, Li Y, *et al*: Whole-genome sequencing reveals diverse models of structural variations in esophageal squamous cell carcinoma. Am J Hum Genet 98: 256-274, 2016.
9. Cheng C, Cui H, Zhang L, Jia Z, Song B, Wang F, Li Y, Liu J, Kong P, Shi R, *et al*: Genomic analyses reveal FAM84B and the NOTCH pathway are associated with the progression of esophageal squamous cell carcinoma. Gigascience 5: 1, 2016.
10. Cui Y, Chen H, Xi R, Cui H, Zhao Y, Xu E, Yan T, Lu X, Huang F, Kong P, *et al*: Whole-genome sequencing of 508 patients identifies key molecular features associated with poor prognosis in esophageal squamous cell carcinoma. Cell Res 30: 902-913, 2020.
11. Lian Y, Niu X, Cai H, Yang X, Ma H, Ma S, Zhang Y and Chen Y: Clinicopathological significance of c-MYC in esophageal squamous cell carcinoma. Tumour Biol 39: 1010428317715804, 2017.
12. Yan T, Cui H, Zhou Y, Yang B, Kong P, Zhang Y, Liu Y, Wang B, Cheng Y, Li J, *et al*: Multi-region sequencing unveils novel actionable targets and spatial heterogeneity in esophageal squamous cell carcinoma. Nat Commun 10: 1670, 2019.
13. Lin DC, Wang MR and Koeffler HP: Targeting genetic lesions in esophageal cancer. Cell Cycle 13: 2013-2014, 2014.
14. Almal SH and Padh H: Implications of gene copy-number variation in health and diseases. J Hum Genet 57: 6-13, 2012.
15. Conrad DF, Pinto D, Redon R, Feuk L, Gokcumen O, Zhang Y, Aerts J, Andrews TD, Barnes C, Campbell P, *et al*: Origins and functional impact of copy number variation in the human genome. Nature 464: 704-712, 2010.

16. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, *et al*: Global variation in copy number in the human genome. Nature 444: 444-454, 2006.
17. Zhang F, Gu W, Hurles ME and Lupski JR: Copy number variation in human health, disease, and evolution. Annu Rev Genomics Hum Genet 10: 451-481, 2009.
18. Lupski JR and Stankiewicz P: Genomic disorders: Molecular mechanisms for rearrangements and conveyed phenotypes. PLoS Genet 1: e49, 2005.
19. Lieber MR, Ma Y, Pannicke U and Schwarz K: Mechanism and regulation of human non-homologous DNA end-joining. Nat Rev Mol Cell Biol 4: 712-720, 2003.
20. Lieber MR: The mechanism of human nonhomologous DNA end joining. J Biol Chem 283: 1-5, 2008.
21. Lee JA, Carvalho CMB and Lupski JR: A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. Cell 131: 1235-1247, 2007.
22. Kazazian HH Jr and Moran JV: The impact of L1 retrotransposons on the human genome. Nat Genet 19: 19-24, 1998.
23. Zhang S, Lu Z, Unruh AK, Ivan C, Baggerly KA, Calin GA, Li Z, Bast RC Jr and Le XF: Clinically relevant microRNAs in ovarian cancer. Mol Cancer Res 13: 393-401, 2015.
24. Zucman-Rossi J, Villanueva A, Nault JC and Llovet JM: Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology 149: 1226-1239.e4, 2015.
25. Wang H, Liang L, Fang JY and Xu J: Somatic gene copy number alterations in colorectal cancer: New quest for cancer drivers and biomarkers. Oncogene 35: 2011-2019, 2016.
26. Choi W, Ochoa A, McConkey DJ, Aine M, Höglund M, Kim WY, Real FX, Kiltie AE, Milsom I, Dyrskjøt L and Lerner SP: Genetic alterations in the molecular subtypes of bladder cancer: Illustration in the cancer genome atlas dataset. Eur Urol 72: 354-365, 2017.
27. Berger AC, Korkut A, Kanchi RS, Hegde AM, Lenoir W, Liu W, Liu Y, Fan H, Shen H, Ravikumar V, *et al*: A comprehensive pan-cancer molecular study of gynecologic and breast cancers. Cancer Cell 33: 690-705.e9, 2018.
28. Kleinjan DA and van Heyningen V: Long-range control of gene expression: Emerging mechanisms and disruption in disease. Am J Hum Genet 76: 8-32, 2005.
29. Girirajan S, Campbell CD and Eichler EE: Human copy number variation and complex genetic disease. Annu Rev Genet 45: 203-226, 2011.
30. Meyerson M, Gabriel S and Getz G: Advances in understanding cancer genomes through second-generation sequencing. Nat Rev Genet 11: 685-696, 2010.
31. Alkan C, Coe BP and Eichler EE: Genome structural variation discovery and genotyping. Nat Rev Genet 12: 363-376, 2011.
32. Mamanova L, Coffey AJ, Scott CE, Kozarewa I, Turner EH, Kumar A, Howard E, Shendure J and Turner DJ: Target-enrichment strategies for next-generation sequencing. Nat Methods 7: 111-118, 2010.
33. Ari Å and Arikan M: Next-generation sequencing: Advantages, disadvantages, and future. In: Plant omics: Trends and applications. Springer, Berlin, pp109-135, 2016.
34. Ogawa A, Celikkol-Aydin S, Gaylarde C, Baptista-Neto JA and Beech I: Microbiomes of biofilms on decorative siliceous stone: Drawbacks and advantages of next generation sequencing. Curr Microbiol 74: 848-853, 2017.
35. Berná L, Rodriguez M, Chiribao ML, Parodi-Talice A, Pita S, Rijo G, Alvarez-Valin F and Robello C: Expanding an expanded genome: Long-read sequencing of Trypanosoma cruzi. Microb Genom 4: e000177, 2018.
36. Lin DC, Hao JJ, Nagata Y, Xu L, Shang L, Meng X, Sato Y, Okuno Y, Varela AM, Ding LW, *et al*: Genomic and molecular characterization of esophageal squamous cell carcinoma. Nat Genet 46: 467-473, 2014.
37. Shi ZZ, Shang L, Jiang YY, Hao JJ, Zhang Y, Zhang TT, Lin DC, Liu SG, Wang BS, Gong T, *et al*: Consistent and differential genetic aberrations between esophageal dysplasia and squamous cell carcinoma detected by array comparative genomic hybridization. Clin Cancer Res 19: 5867-5878, 2013.
38. Qin HD, Liao XY, Chen YB, Huang SY, Xue WQ, Li FF, Ge XS, Liu DQ, Cai Q, Long J, *et al*: Genomic characterization of esophageal squamous cell carcinoma reveals critical genes underlying tumorigenesis and poor prognosis. Am J Hum Genet 98: 709-727, 2016.
39. Lin DC, Wang MR and Koeffler HP: Genomic and epigenomic aberrations in esophageal squamous cell carcinoma and implications for patients. Gastroenterology 154: 374-389, 2018.
40. Huang R, Dai Q, Yang R, Duan Y, Zhao Q, Haybaeck J and Yang Z: A review: PI3K/AKT/mTOR signaling pathway and its regulated eukaryotic translation initiation factors may be a potential therapeutic target in esophageal squamous cell carcinoma. Front Oncol 12: 817916, 2022.
41. Zang W, Wang T, Wang Y, Chen X, Du Y, Sun Q, Li M, Dong Z and Zhao G: Knockdown of long non-coding RNA TP73-AS1 inhibits cell proliferation and induces apoptosis in esophageal squamous cell carcinoma. Oncotarget 7: 19960-19974, 2016.
42. Liu J, Liu ZX, Wu QN, Lu YX, Wong CW, Miao L, Wang Y, Wang Z, Jin Y, He MM, *et al*: Long noncoding RNA AGPG regulates PFKFB3-mediated tumor glycolytic reprogramming. Nat Commun 11: 1507, 2020.
43. Liu X, Zhang M, Ying S, Zhang C, Lin R, Zheng J, Zhang G, Tian D, Guo Y, Du C, *et al*: Genetic alterations in esophageal tissues from squamous dysplasia to carcinoma. Gastroenterology 153: 166-177, 2017.
44. Ma S, Paiboonrungruan C, Yan T, Williams KP, Major MB and Chen XL: Targeted therapy of esophageal squamous cell carcinoma: The NRF2 signaling pathway as target. Ann N Y Acad Sci 1434: 164-172, 2018.
45. Shen H, Yang Y, Xia S, Rao B, Zhang J and Wang J: Blockage of Nrf2 suppresses the migration and invasion of esophageal squamous cell carcinoma cells in hypoxic microenvironment. Dis Esophagus 27: 685-692, 2014.
46. Kawasaki Y, Okumura H, Uchikado Y, Kita Y, Sasaki K, Owaki T, Ishigami S and Natsugoe S: Nrf2 is useful for predicting the effect of chemoradiation therapy on esophageal squamous cell carcinoma. Ann Surg Oncol 21: 2347-2352, 2014.
47. Shibata T, Kokubu A, Saito S, Narisawa-Saito M, Sasaki H, Aoyagi K, Yoshimatsu Y, Tachimori Y, Kushima R, Kiyono T and Yamamoto M: NRF2 mutation confers malignant potential and resistance to chemoradiation therapy in advanced esophageal squamous cancer. Neoplasia 13: 864-873, 2011.
48. Bollong MJ, Yun H, Sherwood L, Woods AK, Lairson LL and Schultz PG: A small molecule inhibits deregulated NRF2 transcriptional activity in cancer. ACS Chem Biol 10: 2193-2198, 2015.
49. Singh A, Venkannagari S, Oh KH, Zhang YQ, Rohde JM, Liu L, Nimmagadda S, Sudini K, Brimacombe KR, Gajghate S, *et al*: Small molecule inhibitor of NRF2 selectively intervenes therapeutic resistance in KEAP1-deficient NSCLC tumors. ACS Chem Biol 11: 3214-3225, 2016.
50. Li Y, Zhu CL, Nie CJ, Li JC, Zeng TT, Zhou J, Chen J, Chen K, Fu L, Liu H, *et al*: Investigation of tumor suppressing function of CACNA2D3 in esophageal squamous cell carcinoma. PLoS One 8: e60027, 2013.
51. Nie C, Qin X, Li X, Tian B, Zhao Y, Jin Y, Li Y, Wang Q, Zeng D, Hong A and Chen X: CACNA2D3 enhances the chemosensitivity of esophageal squamous cell carcinoma to cisplatin via inducing Ca^{2+}-mediated apoptosis and suppressing PI3K/Akt pathways. Front Oncol 9: 185, 2019.
52. Li L, Xu J, Qiu G, Ying J, Du Z, Xiang T, Wong KY, Srivastava G, Zhu XF, Mok TS, *et al*: Epigenomic characterization of a p53-regulated 3p22.2 tumor suppressor that inhibits STAT3 phosphorylation via protein docking and is frequently methylated in esophageal and other carcinomas. Theranostics 8: 61-77, 2018.
53. Li Y, Chen L, Nie CJ, Zeng TT, Liu H, Mao X, Qin Y, Zhu YH, Fu L and Guan XY: Downregulation of RBMS3 is associated with poor prognosis in esophageal squamous cell carcinoma. Cancer Res 71: 6106-6115, 2011.
54. Tang H, Jiang L, Zhu C, Liu R, Wu Y, Yan Q, Liu M, Jia Y, Chen J, Qin Y, *et al*: Loss of cell adhesion molecule L1 like promotes tumor growth and metastasis in esophageal squamous cell carcinoma. Oncogene 38: 3119-3133, 2019.
55. Sugita M, Tanaka N, Davidson S, Sekiya S, Varella-Garcia M, West J, Drabkin HA and Gemmill RM: Molecular definition of a small amplification domain within 3q26 in tumors of cervix, ovary, and lung. Cancer Genet Cytogenet 117: 9-18, 2000.
56. Yang YL, Chu JY, Luo ML, Wu YP, Zhang Y, Feng YB, Shi ZZ, Xu X, Han YL, Cai Y, *et al*: Amplification of PRKCI, located in 3q26, is associated with lymph node metastasis in esophageal squamous cell carcinoma. Genes Chromosomes Cancer 47: 127-136, 2008.
57. Wu Y, Liu X, Hu L, Tao H, Guan X, Zhang K, Bai Y and Yang K: Copy number loss of variation_91720 in PIK3CA predicts risk of esophageal squamous cell carcinoma. Int J Clin Exp Pathol 8: 14479-14485, 2015.

58. Wang P, Shan L, Xue L, Zheng B and Lu N: Genome wide copy number analyses of superficial esophageal squamous cell carcinoma with and without metastasis. Oncotarget 8: 5069-5080, 2017.
59. Li B, Cheung PY, Wang X, Tsao SW, Ling MT, Wong YC and Cheung AL: Id-1 activation of PI3K/Akt/NFkappaB signaling pathway and its significance in promoting survival of esophageal cancer cells. Carcinogenesis 28: 2313-2320, 2007.
60. Gen Y, Yasui K, Zen Y, Zen K, Dohi O, Endo M, Tsuji K, Wakabayashi N, Itoh Y, Naito Y, *et al*: SOX2 identified as a target gene for the amplification at 3q26 that is frequently detected in esophageal squamous cell carcinoma. Cancer Genet Cytogenet 202: 82-93, 2010.
61. Gen Y, Yasui K, Nishikawa T and Yoshikawa T: SOX2 promotes tumor growth of esophageal squamous cell carcinoma through the AKT/mammalian target of rapamycin complex 1 signaling pathway. Cancer Sci 104: 810-816, 2013.
62. Wang X, Ge X, Wang H, Huang J, Song Q, Xu C, Jiang Z, Su J, Wang H, Tan L, *et al*: SOX2 amplification and chromosome 3 gain significantly impact prognosis in esophageal squamous cell carcinoma. Ann Transl Med 9: 321, 2021.
63. Gao H, Teng C, Huang W, Peng J and Wang C: SOX2 promotes the epithelial to mesenchymal transition of esophageal squamous cells by modulating slug expression through the activation of STAT3/HIF-α Signaling. Int J Mol Sci 16: 21643-21657, 2015.
64. Chen B, Liu S, Gan L, Wang J, Hu B, Xu H, Tong R, Yang H, Cristina I, Xue J, *et al*: FGFR1 signaling potentiates tumor growth and predicts poor prognosis in esophageal squamous cell carcinoma patients. Cancer Biol Ther 19: 76-86, 2018.
65. Guagnano V, Kauffmann A, Wöhrle S, Stamm C, Ito M, Barys L, Pornon A, Yao Y, Li F, Zhang Y, *et al*: FGFR genetic alterations predict for sensitivity to NVP-BGJ398, a selective pan-FGFR inhibitor. Cancer Discov 2: 1118-1133, 2012.
66. von Loga K, Kohlhaussen J, Burkhardt L, Simon R, Steurer S, Burdak-Rothkamm S, Jacobsen F, Sauter G and Krech T: FGFR1 amplification is often homogeneous and strongly linked to the squamous cell carcinoma subtype in esophageal carcinoma. PLoS One 10: e0141867, 2015.
67. Luo H, Quan J, Xiao H, Luo J, Zhang Q, Pi G, Ye Y, He R, Liu Y, Su X, *et al*: FGFR inhibitor AZD4547 can enhance sensitivity of esophageal squamous cell carcinoma cells with epithelial-mesenchymal transition to gefitinib. Oncol Rep 39: 2270-2278, 2018.
68. Huang J, Jiang D, Zhu T, Wang Y, Wang H, Wang Q, Tan L, Zhu H, Yao J and Hou Y: Prognostic significance of c-MYC amplification in esophageal squamous cell carcinoma. Ann Thorac Surg 107: 436-443, 2019.
69. Zhang HF, Wu C, Alshareef A, Gupta N, Zhao Q, Xu XE, Jiao JW, Li EM, Xu LY and Lai R: The PI3K/AKT/c-MYC axis promotes the acquisition of cancer stem-like features in esophageal squamous cell carcinoma. Stem Cells 34: 2040-2051, 2016.
70. Li W, Zhang L, Guo B, Deng J, Wu S, Li F, Wang Y, Lu J and Zhou Y: Exosomal FMR1-AS1 facilitates maintaining cancer stem-like cell dynamic equilibrium via TLR7/NFκB/c-Myc signaling in female esophageal carcinoma. Mol Cancer 18: 22, 2019.
71. Wang Y, Cheng J, Xie D, Ding X, Hou H, Chen X, Er P, Zhang F, Zhao L, Yuan Z, *et al*: NS1-binding protein radiosensitizes esophageal squamous cell carcinoma by transcriptionally suppressing c-Myc. Cancer Commun (Lond) 38: 33, 2018.
72. Yang J, Kong P, Yang J, Jia Z, Hu X, Wang Z, Cui H, Bi Y, Qian Y, Li H, *et al*: High TSTA3 expression as a candidate biomarker for poor prognosis of patients with ESCC. Technol Cancer Res Treat 17: 533033818781405, 2018.
73. Zhang L, Gao Y, Zhang X, Guo M, Yang J, Cui H, Kong P, Niu X, Bi Y, Xu J, *et al*: TSTA3 facilitates esophageal squamous cell carcinoma progression through regulating fucosylation of LAMP2 and ERBB2. Theranostics 10: 11339-11358, 2020.
74. Lin X, Yan C, Gao Y, Du J, Zhu X, Yu F, Huang T, Dai J, Ma H, Jiang Y, *et al*: Genetic variants at 9p21.3 are associated with risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Sci 108: 250-255, 2017.
75. Shen TY, Mei LL, Qiu YT and Shi ZZ: Identification of candidate target genes of genomic aberrations in esophageal squamous cell carcinoma. Oncol Lett 12: 2956-2961, 2016.
76. Wang Q, Bai J, Abliz A, Liu Y, Gong K, Li J, Shi W, Pan Y, Liu F, Lai S, *et al*: An old story retold: Loss of G1 control defines a distinct genomic subtype of esophageal squamous cell carcinoma. Genomics Proteomics Bioinformatics 13: 258-270, 2015.
77. Su D, Zhang D, Jin J, Ying L, Han M, Chen K, Li B, Wu J, Xie Z, Zhang F, *et al*: Identification of predictors of drug sensitivity using patient-derived models of esophageal squamous cell carcinoma. Nat Commun 10: 5076, 2019.
78. Clark ES, Brown B, Whigham AS, Kochaishvili A, Yarbrough WG and Weaver AM: Aggressiveness of HNSCC tumors depends on expression levels of cortactin, a gene in the 11q13 amplicon. Oncogene 28: 431-444, 2009.
79. Kwek SS, Roy R, Zhou H, Climent J, Martinez-Climent JA, Fridlyand J and Albertson DG: Co-amplified genes at 8p12 and 11q13 in breast tumors cooperate with two major pathways in oncogenesis. Oncogene 28: 1892-1903, 2009.
80. Huang J, Song Q, Wang H, Wang H, Xu C, Wang X, Jiang Z, Wang Y, Xu Y, Su J, *et al*: Poor prognostic impact of FGF4 amplification in patients with esophageal squamous cell carcinoma. Hum Pathol 80: 210-218, 2018.
81. Yu Y, Cao J, Wu W, Zhu Q, Tang Y, Zhu C, Dai J, Li Z, Wang J, Xue L, *et al*: Genome-wide copy number variation analysis identified ANO1 as a novel oncogene and prognostic biomarker in esophageal squamous cell cancer. Carcinogenesis 40: 1198-1208, 2019.
82. Shang L, Hao JJ, Zhao XK, He JZ, Shi ZZ, Liu HJ, Wu LF, Jiang YY, Shi F, Yang H, *et al*: ANO1 protein as a potential biomarker for esophageal cancer prognosis and precancerous lesion development prediction. Oncotarget 7: 24374-24382, 2016.
83. Zhang W, Hong R, Li L, Wang Y, Du P, Ou Y, Zhao Z, Liu X, Xiao W, Dong D, *et al*: The chromosome 11q13.3 amplification associated lymph node metastasis is driven by miR-548k through modulating tumor microenvironment. Mol Cancer 17: 125, 2018.
84. Dong G, Mao Q, Yu D, Zhang Y, Qiu M, Dong G, Chen Q, Xia W, Wang J, Xu L and Jiang F: Integrative analysis of copy number and transcriptional expression profiles in esophageal cancer to identify a novel driver gene for therapy. Sci Rep 7: 42060, 2017.
85. Sawada R, Maehara R, Oshikiri T, Nakamura T, Itoh T, Kodama Y, Kakeji Y and Zen Y: MDM2 copy number increase: A poor prognostic, molecular event in esophageal squamous cell carcinoma. Hum Pathol 89: 1-9, 2019.
86. Xiao FK, Guo S, Yang F, Zhao LS and Wang LD: MDM2 and its functional polymorphism SNP309 contribute to the development of esophageal carcinoma. J Gene Med 21: e3086, 2019.
87. He T, Guo J, Song H, Zhu H, Di X, Min H, Wang Y, Chen G, Dai W, Ma J, *et al*: Nutlin-3, an antagonist of MDM2, enhances the radiosensitivity of esophageal squamous cancer with wild-type p53. Pathol Oncol Res 24: 75-81, 2018.
88. Okamoto H, Fujishima F, Kamei T, Nakamura Y, Ozawa Y, Miyata G, Nakano T, Katsura K, Abe S, Taniyama Y, *et al*: Murine double minute 2 predicts response of advanced esophageal squamous cell carcinoma to definitive chemoradiotherapy. BMC Cancer 15: 208, 2015.
89. Sun Y, Shi N, Lu H, Zhang J, Ma Y, Qiao Y, Mao Y, Jia K, Han L, Liu F, *et al*: ABCC4 copy number variation is associated with susceptibility to esophageal squamous cell carcinoma. Carcinogenesis 35: 1941-1950, 2014.
90. Yasui K, Imoto I, Fukuda Y, Pimkhaokham A, Yang ZQ, Naruto T, Shimada Y, Nakamura Y and Inazawa J: Identification of target genes within an amplicon at 14q12-q13 in esophageal squamous cell carcinoma. Genes Chromosomes Cancer 32: 112-118, 2001.
91. Sano M, Aoyagi K, Takahashi H, Kawamura T, Mabuchi T, Igaki H, Tachimori Y, Kato H, Ochiai A, Honda H, *et al*: Forkhead box A1 transcriptional pathway in KRT7-expressing esophageal squamous cell carcinomas with extensive lymph node metastasis. Int J Oncol 36: 321-330, 2010.
92. Xu Y, Wang W, Li L, Liu J, Wu X, Yu J, Wang H, Cui W and Zhang R: FOXA1 and CK7 expression in esophageal squamous cell carcinoma and its prognostic significance. Neoplasma 65: 469-476, 2018.
93. Bi Y, Guo S, Xu X, Kong P, Cui H, Yan T, Ma Y, Cheng Y, Chen Y, Liu X, *et al*: Decreased ZNF750 promotes angiogenesis in a paracrine manner via activating DANCR/miR-4707-3p/FOXC2 axis in esophageal squamous cell carcinoma. Cell Death Dis 11: 296, 2020.
94. Kong P, Xu E, Bi Y, Xu X, Liu X, Song B, Zhang L, Cheng C, Yan T, Qian Y, *et al*: Novel ESCC-related gene ZNF750 as potential prognostic biomarker and inhibits epithelial-mesenchymal transition through directly depressing SNAI1 promoter in ESCC. Theranostics 10: 1798-1813, 2020.

95. Du Plessis L, Dietzsch E, Van Gele M, Van Roy N, Van Helden P, Parker MI, Mugwanya DK, De Groot M, Marx MP, Kotze MJ and Speleman F: Mapping of novel regions of DNA gain and loss by comparative genomic hybridization in esophageal carcinoma in the black and colored populations of South Africa. Cancer Res 59: 1877-1883, 1999.
96. Gorringe KL, Ramakrishna M, Williams LH, Sridhar A, Boyle SE, Bearfoot JL, Li J, Anglesio MS and Campbell IG: Are there any more ovarian tumor suppressor genes? A new perspective using ultra high-resolution copy number and loss of heterozygosity analysis. Genes Chromosomes Cancer 48: 931-942, 2009.
97. Girard L, Zöchbauer-Müller S, Virmani AK, Gazdar AF and Minna JD: Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non-small cell lung cancer, and loci clustering. Cancer Res 60: 4894-4906, 2000.
98. Zhu YH, Fu L, Chen L, Qin YR, Liu H, Xie F, Zeng T, Dong SS, Li J, Li Y, *et al*: Downregulation of the novel tumor suppressor DIRAS1 predicts poor prognosis in esophageal squamous cell carcinoma. Cancer Res 73: 2298-2309, 2013.
99. Qin H, Li Y, Zhang H, Wang F, He H, Bai X and Li S: Prognostic implications and oncogenic roles of MYBL2 protein expression in esophageal squamous-cell carcinoma. Onco Targets Ther 12: 1917-1927, 2019.
100. Chong CR and Jänne PA: The quest to overcome resistance to EGFR-targeted therapies in cancer. Nat Med 19: 1389-1400, 2013.
101. Ciardiello F and Tortora G: EGFR antagonists in cancer treatment. N Engl J Med 358: 1160-1174, 2008.
102. Ruhstaller T, Thuss-Patience P, Hayoz S, Schacher S, Knorrenschild JR, Schnider A, Plasswilm L, Budach W, Eisterer W, Hawle H, *et al*: Neoadjuvant chemotherapy followed by chemoradiation and surgery with and without cetuximab in patients with resectable esophageal cancer: A randomized, open-label, phase III trial (SAKK 75/08). Ann Oncol 29: 1386-1393, 2018.
103. Han X, Lu N, Pan Y and Xu J: Nimotuzumab combined with chemotherapy is a promising treatment for locally advanced and metastatic esophageal cancer. Med Sci Monit 23: 412-418, 2017.
104. Olsson AK, Dimberg A, Kreuger J and Claesson-Welsh L: VEGF receptor signalling-in control of vascular function. Nat Rev Mol Cell Biol 7: 359-371, 2006.
105. Li J and Wang L: Efficacy and safety of apatinib treatment for advanced esophageal squamous cell carcinoma. Onco Targets Ther 10: 3965-3969, 2017.
106. Zhang B, Qi L, Wang X, Xu J, Liu Y, Mu L, Wang X, Bai L and Huang J: Phase II clinical trial using camrelizumab combined with apatinib and chemotherapy as the first-line treatment of advanced esophageal squamous cell carcinoma. Cancer Commun (Lond) 40: 711-720, 2020.
107. Xu M, Huang H, Xiong Y, Peng B, Zhou Z, Wang D and Yang X: Combined chemotherapy plus endostar with sequential stereotactic radiotherapy as salvage treatment for recurrent esophageal cancer with severe dyspnea: A case report and review of the literature. Oncol Lett 8: 291-294, 2014.
108. Waks AG and Winer EP: Breast cancer treatment: A review. JAMA 321: 288-300, 2019.
109. Hecht JR, Bang YJ, Qin SK, Chung HC, Xu JM, Park JO, Jeziorski K, Shparyk Y, Hoff PM, Sobrero A, *et al*: Lapatinib in combination with capecitabine plus oxaliplatin in human epidermal growth factor receptor 2-positive advanced or metastatic gastric, esophageal, or gastroesophageal adenocarcinoma: TRIO-013/LOGiC-a randomized phase III trial. J Clin Oncol 34: 443-451, 2016.
110. Hirata H, Niida A, Kakiuchi N, Uchi R, Sugimachi K, Masuda T, Saito T, Kageyama SI, Motomura Y, Ito S, *et al*: The evolving genomic landscape of esophageal squamous cell carcinoma under chemoradiotherapy. Cancer Res 81: 4926-4938, 2021.
111. Batista PJ and Chang HY: Long noncoding RNAs: Cellular address codes in development and disease. Cell 152: 1298-1307, 2013.
112. Flynn RA and Chang HY: Long noncoding RNAs in cell-fate programming and reprogramming. Cell Stem Cell 14: 752-761, 2014.
113. Sugimura K, Miyata H, Tanaka K, Hamano R, Takahashi T, Kurokawa Y, Yamasaki M, Nakajima K, Takiguchi S, Mori M and Doki Y: Let-7 expression is a significant determinant of response to chemotherapy through the regulation of IL-6/STAT3 pathway in esophageal squamous cell carcinoma. Clin Cancer Res 18: 5144-5153, 2012.
114. Huang L, Wang Y, Chen J, Wang Y, Zhao Y, Wang Y, Ma Y, Chen X, Liu W, Li Z, *et al*: Long noncoding RNA PCAT1, a novel serum-based biomarker, enhances cell growth by sponging miR-326 in oesophageal squamous cell carcinoma. Cell Death Dis 10: 513, 2019.
115. Hu L, Wu Y, Tan D, Meng H, Wang K, Bai Y and Yang K: Up-regulation of long noncoding RNA MALAT1 contributes to proliferation and metastasis in esophageal squamous cell carcinoma. J Exp Clin Cancer Res 34: 7, 2015.
116. Li Z, Zhou Y, Tu B, Bu Y, Liu A and Kong J: Long noncoding RNA MALAT1 affects the efficacy of radiotherapy for esophageal squamous cell carcinoma by regulating Cks1 expression. J Oral Pathol Med 46: 583-590, 2017.
117. Azizi E, Carr AJ, Plitas G, Cornish AE, Konopacki C, Prabhakaran S, Nainys J, Wu K, Kiseliovas V, Setty M, *et al*: Single-cell map of diverse immune phenotypes in the breast tumor microenvironment. Cell 174: 1293-1308.e36, 2018.
118. Gao S, Yan L, Wang R, Li J, Yong J, Zhou X, Wei Y, Wu X, Wang X, Fan X, *et al*: Tracing the temporal-spatial transcriptome landscapes of the human fetal digestive tract using single-cell RNA-sequencing. Nat Cell Biol 20: 721-734, 2018.
119. Xiao Z, Dai Z and Locasale JW: Metabolic landscape of the tumor microenvironment at single cell resolution. Nat Commun 10: 3763, 2019.
120. Zong C, Lu S, Chapman AR and Xie XS: Genome-wide detection of single-nucleotide and copy-number variations of a single human cell. Science 338: 1622-1626, 2012.
121. Vitak SA, Torkenczy KA, Rosenkrantz JL, Fields AJ, Christiansen L, Wong MH, Carbone L, Steemers FJ and Adey A: Sequencing thousands of single-cell genomes with combinatorial indexing. Nat Methods 14: 302-308, 2017.
122. Chen Z, Zhao M, Liang J, Hu Z, Huang Y, Li M, Pang Y, Lu T, Sui Q, Zhan C, *et al*: Dissecting the single-cell transcriptome network underlying esophagus non-malignant tissues and esophageal squamous cell carcinoma. EBioMedicine 69: 103459, 2021.