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Genomic and Epigenomic Aberrations in Esophageal Squamous Cell Carcinoma and Implications for Patients

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Abstract

Esophageal squamous cell carcinoma (ESCC) is a common malignancy without effective therapy. The exomes of more than 600 ESCCs have been sequenced in the past 4 years, and numerous key aberrations have been identified. Recently, researchers reported both inter- and intratumor heterogeneity. Although these are interesting observations, their clinical implications are unclear due to the limited number of samples profiled. Epigenomic alterations, such as changes in DNA methylation, histone acetylation, and RNA editing, also have been observed in ESCCs. However, it is not clear what proportion of ESCC cells carry these epigenomic aberrations or how they contribute to tumor development. We review the genomic and epigenomic characteristics of ESCCs, with a focus on emerging themes. We discuss their clinical implications and future research directions.

Keywords

Esophageal Cancer; Genomics; Epigenomics

Esophageal cancer is the sixth leading cause of cancer-related mortality worldwide,[1,2] with more than 480,000 new cases diagnosed yearly.[2] More than 80% of esophageal cancers are esophageal squamous cell carcinomas (ESCCs).[3] With the advent of new biochemical technologies (particularly next-generation sequencing), hundreds of ESCCs have been profiled in a comprehensive and unbiased manner using whole-exome sequencing (WES) in the past 4 years. Our understanding of the genomic features of this cancer has therefore greatly advanced.

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Conflicts of interest

The authors disclose no conflicts.

Genomic analyses provide evidence for pervasive abnormalities in the ESCC epigenome: epigenetic regulators themselves are frequently altered by genetic changes. Although the ESCC epigenome per se has not been characterized as extensively as its genome, many epigenetic dys-regulations have been recently discovered, and their biologic significance determined. However, these advances in our understanding of the molecular alterations in ESCC have not been translated to the bedside.

There is no sensitive method for early detection of ESCC, so more than half of patients with these tumors have distal metastases at the time of diagnosis; only 10% to 20% survive for 5 years.[3] Several tumor types have genetic alterations that can be targeted therapeutically, such as $HER2^+$ breast tumors, $EGFR^+$ lung tumors, and $BRAF^+$ melanomas, but such actionable alterations have not been identified in ESCCs. We review genomic and epigenomic aberrations found in ESCC, and discuss how these findings have increased our understanding of ESCC pathogenesis and progression. These acquired lesions might be used as biomarkers or targeted by therapeutic agents.

Somatic Alterations in ESCC Genomes

Copy Number Alterations and Structural Rearrangements

Chromosome aneuploidy and arm-level aberrations in ESCCs, discovered using traditional approaches (such as karyotyping and fluorescence in situ hybridization), have been summarized elsewhere.[4] We focus on focal copy number alterations (CNAs) and structural rearrangements identified by high-resolution and high-throughput methods, including single-nucleotide polymorphism microarrays and next-generation sequencing.

Compared with aneuploidy and arm-level alterations, focal CNAs have a higher probability of making changes to genes that provide cancer cells with a proliferative advantage.[5,6] The most frequent high-level amplifications and homozygous deletions involve 11q13.2–q13.3 and 9p21.3 (locus of *CDKN2A* and *CDKN2B*), respectively (Table 1). Copy number gains increase levels of mRNAs transcribed from more than 80% of genes situated at 11q13.[7] These include oncogenes, such as *CPT1A*, *ANO1*, *ORAOV1*, *CCND1*, *FGF3, FGF4, FGF19*, *CTTN*, and *MIR548K*; their contributions to the malignant phenotype of ESCC cells have been verified.[8–11] The genomic material between 11q13.2 and 11q13.3 is nonuniformly increased, with the most recurrent peak spanning *CCND1.*[12]

CCND1 is amplified by breakage fusion bridge cycles, which occurs under conditions of chromosomal instability.[13] This gene is often coamplified with its neighboring oncogene *CTTN;* its product promotes migration of ESCC cells.[14] Additional recurrent focal amplifications in ESCC include those at 8p11.23 (*FGFR1*), 8q24.21 (*MYC*), 7p11.2 (*EGFR*), 12p12.1 (*KRAS*), 12q15 (*MDM2*), 3q26 (*TP63* and *PRKCI*), 3q26.32–q26.33 (*SOX2* and *PIK3CA*), and 14q13.3 (*NKX2-1*). Other frequent homozygous deletions contain 2q22.1–q22.2 (*LRP1B*), 9p24.1 (*PTPRD*), and 3p14.2 (*FHIT*). All of these genes have been validated as drivers of development of ESCC or other cancers.[12,13,15–27]

Recently, researchers also have performed high-resolution characterization of structural rearrangements in ESCCs, including intrachromosome insertions, inversions and

duplications, and interchromosome translocations. These have been identified by WGS and associated mathematic analyses.

A total of 62 ESCC genomes have been characterized using the WGS platform, and more than 1000 alterations were identified as structural rearrangements.[8,13,28,29] Most structural rearrangements are not likely to have pathogenic potential, but a few recurrent ones might be candidate driver events, such as frequent structural breakpoints affecting the *KCNB2* gene,[8] or an in-frame fusion between *TRAPPC9* and *CLVS1*. This fusion results from chromothripsis-associated rearrangements, in which thousands of clustered rearrangements occur during a single event in confined genomic regions. Other possible drivers of ESCC development include an in-frame fusion between *EIF3E* and *RAD51B*, caused by interchromosomal translocations.[13] The tumor-promoting effects of TRAPPC9 and RAD51B have been reported in other types of cancers,[30–32] so these rearrangements could have important biological effects in ESCC cells. Additional structural rearrangements involving potential oncogenes included *MYBL2* duplication, fusions of *RUNX1T1–PHACTR1*, *MAML2–TTC28*, *ASXL1–RNF170*, and *FGF19–SHANK2*.[29] Despite the discovery of these interesting rearrangements, the overall number of WGS-profiled ESCC samples is small. Large-scale, more uniformly processed WGSs are needed to identify the genomic rearrangements that contribute to development of ESCC.

Somatic Mutations

Sequence analyses of ESCC tumor–germline paired exomes[6,8,12,29,33–35] identified a total of 22 mutation-associated driver genes, also known as significantly mutated genes (SMGs). Here the significance refers to the functional relevance of the somatic variant, which is measured by bioinformatic methods modeling molecular characteristics of driver and passenger mutations, including rates of silent vs nonsilent mutations, mutation spectrum, gene expression level, and DNA replication time.[36] ESCCs share multiple SMGs exclusively with squamous cell carcinoma of the lung (LUSC)[37] and head and neck squamous cell carcinoma (HNSCC).[38] Some of these regulate squamous cell differentiation, such as *ZNF750* and *NOTCH1*. These findings indicate that genetic aberrations could have similar oncogenic potential in cells of similar origins, with similar gene expression profiles and cell differentiation programs. Therefore, neoplasms that arise from developmentally similar lineages are more molecularly alike than neoplasms that arise from different cell lineages but are located within the same organ. This concept has important clinical implications because tumors with similar molecular features can share bio-markers and therapeutic targets. Design of clinical trials and biomarker development for ESCC may benefit from the studies in LUSC and HNSCC, and vice versa.

Like many other cancer types, ESCCs contain prevalent mutations in *TP53*. Other driver mutations are much less frequent in ESCCs (found in fewer than 20% of samples). Thirteen of the 22 SMGs are consistently identified across different cohorts, and most have been associated with development of other tumor types (Table 2). Of the remaining SMGs, 5 have been validated in functional experiments that confirmed their biological effect, indicating that these genes are drivers of ESCC development.

A total of 10 SMGs have additional genomic or epi-genomic aberrations in ESCCs, suggesting that their activation or inactivation are important for development of ESCC. Mutations in *FAM135B*,[8] *EP300*,[33,34] and *TET2*[34] have been associated with shorter times of patient survival. Nevertheless, the discovery of ESCC SMGs, particularly those mutated at low frequency, is far from saturated because of insufficient statistical power. Specifically, studies of ESCCs typically sequenced the exomes of 100 to 150 tumor-germline pairs, whereas mathematical analysis estimated that 1000 to 2000 samples are needed to identify with confidence SMGs mutated in 2% to 3% of the population, taking into account the background mutational rate of ESCC.[36]

Deconvolution of the complex mutational spectrum with mathematical algorithms has increased our understanding of the mutation process in cancer cells. Endogenous (such as spontaneous deamination of 5-methylcytosines) and exogenous (such as ultraviolet radiation) factors cause DNA damage and induce DNA damage-repair mechanisms, leaving genomic imprints that can be detected by sequence analyses. Mutation spectrums mathematically extracted from these genomic imprints are called mutation signatures. These signatures reflect different biologic perturbations that occur before and during malignant transformation and progression.

Bioinformatics analyses have identified several mutation signatures in ESCCs. Signature 1 is characterized by C > T substitutions at NpCpG trinucleotides and is the most commonly observed signature in ESCC samples. These mutations result from spontaneous deamination of 5-methylcytosine accumulating during a life time.[39] Another clock-like mutation signature found in ESCC is Signature 5, which is characterized by transcriptional strand bias for T > C substitutions at ApTpN trinucleotides.[40] This signature is found in a lower proportion of ESCC samples than signature 1. Signature 2 is characterized by C > T mutations and signature 13 is characterized by C > G mutations[6,12,34,35] at TpCpN. Both signatures 2 and 13 are associated with increased deaminases activity of apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC), which converts cytidine to uracil, and subsequently activates the base excision repair pathway. APOBEC3B activity is higher in ESCCs than nonmalignant esophagus mucosa.[12] Signature 4, characterized by transcription strand bias for C > A substitutions, is found in a small set of ESCC tissues.[41] This signature resembles the mutation pattern generated by benzo[a]pyrene (a tobacco carcinogen) in experimental systems, indicating tobacco exposure in its etiology.

The overall mutation spectrum of ESCC is similar to that of HNSCC,[38] but distinct from esophageal adenocarcinoma, which is associated with gastric acid reflux and has mutation signature 17. This result suggests that lineage-specific mutation processes may contribute to these 2 different histological subtypes of esophageal cancers. Mutations in *PIK3CA* and *ZNF750* were significantly enriched in ESCCs with signature 2 and signature 13,[6,34] suggesting that elevated APOBEC activity may lead to acquisition of driver mutations in these ESCCs. These signature analyses were performed using WES data, so their stability and robustness require large-scale WGS validation studies.

Epigenomic Alterations in ESCCs

Compared with many of the unbiased genomic analysis, most epigenomic studies of ESCCs are based on candidate gene/region approaches. Nevertheless, these approaches have identified many factors that contribute to esophageal carcinogenesis, such as epigenetic silencing of genes, super-enhancer activation, and RNA editing.

DNA Methylation

DNA methylation profiling studies have shown that, like other cancer types, the ESCC genome contains focal areas of hypermethylation and widespread areas of hypo-methylation, compared with nonmalignant esophageal mucosa. These epigenomic aberrations each contribute to the pathogenesis of ESCC, through different mechanisms.

Promoter hypermethylation silences tumor suppressor genes such as *CDKN2A*, *CDKN2B,*[42,43] *DLC1*,[44] *LRP1B*,[26] and *RASSF1A*[45] (Table 3). A few genes encoding microRNAs are also methylated and down-regulated in ESCCs, compared with nonmalignant tissues, including those with anti-proliferative functions, such as microRNA34a[46] and micro-RNA375.[47] It remains to be determined whether some (if any) of these promoter hypermethylation events are specific to ESCC cells.

These methylation patterns might be used in the clinic. For example, *CDKN2A*, *CDKN2B,*[42,43] and *TFF1*[48] are hyper-methylated during early stages of esophageal carcinogenesis, and might therefore serve as biomarkers for early diagnosis of ESCC. In addition, hypermethylation of the *APC*[49] and *FHIT*[50] promoters was significantly associated with reduced survival times of patients with ESCC, so these might be prognostic factors. Plasma samples of patients suspected of having ESCC might be collected and analyzed for these hypermethylation events.[51,52]

Compared with focal hypermethylation, global hypo-methylation is much less understood in ESCC. This is because most methylation studies of ESCCs were conducted using a candidate gene approach. Several groups performed Infinium HumanMethylation450 array profiling, measuring 450,000 individual CpGs across the human genome. However, this array by design covers much more densely gene promoter regions, relative to the rest of the genome.

To estimate global methylation, a few studies have assessed the methylation level of the long interspersed nuclear element-1 (LINE-1) elements as a marker.[53–56] Consistent genome-wide hypomethylation was observed in ESCCs from different cohorts. LINE-1 hypomethylation was associated with increased chromosomal instability, *TP53* mutation, lymph node metastasis,[54] as well as a shorter survival times of patients.[56] However, these associations are poorly understood; alterations of the LINE-1 itself does not provide insights into the impact of methylation changes on functional genomic domains (such as at enhancers, insulators, or silencers). It is important to study these types of changes in cancer research. DNA methylation can alter the ability of transcription factors to bind DNA and regulate gene expression.[57–59] In glioma cells, changes in DNA methylation reduced the binding of CTCF to its motif sequence, disrupting insulated genomic domains.[60] However,

many methylation changes affect distal promoter elements, which cannot be readily determined by promoter-centric approaches. Thus, the ESCC methylome awaits further characterization through sequence-based approaches, such as reduced representation bisulfite sequencing and whole-genome bisulfite sequencing, which detect methylation changes with high resolution and a high level of sensitivity.

Chromatin Modification

Factors that regulate chromatin modification may also affect development of ESCC. Many mutations detected in ESCCs lie in genes encoding proteins that modify DNA/histone (*EP300*, *CREBBP*, and *MLL2*), genes that remove histone modifications (*KDM6A* and *TET2*), and proteins that remodel chromatin structures (*ARID1A*, *ARID2*, and *SMARCC2*). Histone methyltransferases, including EZH2[61] and G9a,[62] are overexpressed in ESCCs in the absence of genomic alterations. High-throughput proteomic analysis and immunohistochemical analysis detected abnormal levels of methylation at histone H3 (H3K27me3)[61,63,64] and histone H4 (H4K79me2)[65] in primary ESCC tissues, compared with nonmalignant tissues. Ectopic expression of EZH2 in ESCC cell lines elevated the overall level of H3K27me3.[61] Increased levels of H3K27me3 have been associated with outcomes of patients with ESCC by 2 independent groups.[61,63]

Acetylation at lysine 27 the histone H3 protein (H3K27ac) is a predictive marker of transcription activation. Genomic regions with extensive H3K27ac modification have been associated with a special group of enhancers known as super-enhancers.[66] Super-enhancers promote transcriptional activation of oncogenes in ESCC cells and squamous lineage-specific genes.[67] It is not clear how alterations of chromatin modification contribute to development of ESCCs. Nonetheless, researchers have begun to investigate the antineoplastic effects of inhibiting the activities of HDAC,[68] CDK7,[67] and LSD1[69] in ESCC cells.

RNA Editing

RNA editing processes are misregulated in ESCCs. In mammalian cells, adenosine deaminases acting on RNA (ADAR) family members, frequently convert adenosine (A) to inosine (I) on RNA molecules.[70] The *ADAR1* gene was reported to be amplified and overexpressed in primary ESCC tissues, and ectopic expression of ADAR1 enhanced a malignant phenotype of ESCC cells. ADAR1 binds to mRNAs, including *AZIN1* and *FLNB*, to mediate the A-to-I editing; this was increased in ESCC tissues compared with nonmalignant esophagus epithelia. This process alters the coding sequence of AZIN1, turning it into an oncogenic protein that promotes cell proliferation, migration, and invasion of ESCC cells.[71]

Intriguingly, another ADAR family member, ADAR2, seems to have opposite functions. ADAR2 is down-regulated in ESCC samples, and its expression from a transgene reduced proliferation of ESCC cells. ADAR2 edits the *IGFBP7* mRNA, leading to stabilization of the IGFBP7 protein, which functions as a tumor suppressor.[72] Further investigation of RNA editing in ESCC is required to determine its role in pathogenesis.

Inter- and Intratumor Genomic and Epigenomic Heterogeneity

ESCC is not 1 disease, and at least 2 forms of tumor heterogeneity exist. Interpatient or intertumor heterogeneity means that ESCC tumors have different biological, clinical, and pathology features among different patients. Intratumor heterogeneity, on the other hand, refers to differences in cancer cells or tissues from the same tumor.

Intertumor Heterogeneity

The intertumor heterogeneity of ESCC has been known for decades. However, unlike several other common cancers (such as leukemia, breast cancer, and lung cancer), which have been subtyped based on biological or molecular features, the intertumor variation in the molecular features of ESCCs was not appreciated until recently.

The Cancer Genome Atlas Consortium performed a comprehensive molecular characterization of 90 ESCCs, using multiple genomic and epigenomic platforms.[35] Matched WES, RNA sequencing, and DNA methylation data identified 2 major subtypes and 1 minor subtype within ESCC (designated as ESCC1, ESCC2, and ESCC3 by the authors). ESCC1 was characterized by enriched genomic aberrations targeting the nuclear factor, erythroid 2 like 2 (NRF2) pathway (*NFE2L2, KEAP1, CUL3,* and *ATG7*), as well as amplifications of *SOX2*, *TP63,* and *YAP1.* ESCC2 was found to be devoid of these genetic alterations, but instead had more frequent mutations or deletions in SMGs such as *KDM6A, MLL2, NOTCH1,* and *ZNF750*. The minor subtype, ESCC3, had *SMARCA4* mutations and fewer CNAs than the ESCC1 or ESCC2 subtypes. However, only 4 individuals were found to have ESCC3 tumors, so the stability and significance of this subtype is unclear.

At the transcriptome level, ESCC1 subtype was analogous to the classical subtype of LUSC and HNSCC, sharing a squamous-cell–specific gene expression program. These subtypes also showed geographic associations; Asian individuals were more likely to have ESCC1 and non-Asian individuals were more likely to have ESCC2.[35] This integrative analysis provided insight into the molecular differences among ESCC from different patients. Large-scale studies are required to validate these findings and determine their clinical significance.

Intratumor Heterogeneity

There have been recent reports from unbiased and precise investigations of ESCC intratumor heterogeneity, made possible by a multiregion deep-sequencing approach.[73] Importantly, 40% of driver mutations were spatially heterogeneous, meaning they could be detected only in some, but not all cancer cells from the same tumor. Mathematical reconstruction of tumor progression showed that these heterogeneous mutations were relatively late events in ESCC development, promoting the expansion of subclones of cancer cells.

Like many other tumor types, in ESCCs, driver genes (*TP53, MLL2, ZNF750*) tend to have more clonal mutations than passenger genes, indicating that they occur early during ESCC tumorigenesis. Nevertheless, despite this tendency, several ESCC oncogenes, such as *PIK3CA, MTOR, NFE2L2*, and *KIT,* become mutated in a subclonal manner. The heterogeneous nature of many driver lesions has important clinical implications in ESCC. For example, drugs that target ubiquitous (early) drivers likely offer more clinical benefit

than those that target heterogeneous (late) ones. In a clinical trial, AZD4547, an inhibitor of fibroblast growth factor receptor (FGFR), reduced growth of only gastric tumors with clonal *FGFR2* amplification, not those with subclonal amplification of this gene.[74] Agents designed to target sub-clonal drivers of multiple myeloma paradoxically stimulated proliferation of cancer cells that did not contain such driver mutations.[75] Intratumor heterogeneity is therefore an important determinant of response to therapy.

Intratumor heterogeneity exists in ESCCs beyond the level of somatic mutations. Other important genomic and epigenomic variations, including copy number and DNA methylation changes, contribute to the spatial diversity within single ESCC tumors. Degrees of intratumor heterogeneity and tumor evolution determined by DNA methylation recapitulate those determined by somatic mutations, indicating interactions between genomic and epigenomic events during development of ESCC.[73] Similar findings have also been reported from prostate tumors[76] and gliomas.[77]

A higher degree of genomic intratumor heterogeneity was associated with worse outcomes of patients with HNSCC.[78] Using reduced representation bisulfite sequencing to profile chronic lymphocytic leukemia, Landau et al[79] demonstrated that the degree of intratumor heterogeneity of methylation correlated with shorter time of relapse-free survival. Intratumor heterogeneity of mutation and methylation also associate with outcomes of patients with hepatocellular carcinoma.[80]

Many additional features of ESCC intratumor heterogeneity and its biological significance remain to be explored. First, ESCCs from only 15 patients have been profiled[73,81]; more cases are required to determine patterns of ESCC clonal architecture and tumor progression. Longitudinal studies of matched primary and recurrent or metastatic tumors will identify drug-resistant and metastasis-initiating clones. Single-cell sequence analyses are required to uncover the ultimate amount of heterogeneity among cells in a tumor. This approach also will shed light on the mechanisms of ESCC evolution: do they follow the laws of natural selection,[82] neutral growth,[83] or both?

Progression of Esophageal Squamous Precancerous Lesions to ESCC

The stepwise progression from normal squamous epithelium to ESCC can be traced by histologic analysis, from basal cell hyperplasia (BCH), to mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma in situ (CIS), and invasive carcinoma.[84,85] Data from long-term follow-up studies of patients (for more than 10 years) confirmed that squamous dysplasia, detected by histologic analysis, is the only precursor lesion to ESCC.[84] In 2010, the World Health Organization revised the classification, in which mild dysplasia to CIS are called intraepithelial neoplasia (IEN), mild dysplasia and moderate dysplasia classified as low-grade IEN (LGIEN), and severe dysplasia or CIS called high-grade IEN (HGIEN).[86] Patients with HGIEN are treated with endoscopic mucosal resection, and patients with LGIEN are followed.[87] Patients with HGIEN are often overtreated, whereas those with LGIEN are undertreated. Thus, stratification systems are therefore needed to better determine cancer risk of patients with IEN.

Over the past decade, genomic and epigenomic factors have been associated with transformation of esophageal squamous precancerous lesions into ESCC (Figure 1). Alterations detected in BCH, the initiating step, have not been profiled robustly because it is difficult to obtain samples. Although *TP53* mutations were identified in BCH more than 2 decades ago,[88] in vivo studies demonstrated that this mutation alone was insufficient for development of invasive cancer[89]; additional lesion(s) are required.

It has been a challenge to identify lesions in BCH that lead to tumorigenesis until recently. Liu et al[90] performed WGS and targeted sequencing analyses of matched BCH, IEN, and ESCC samples from 70 individuals. As expected, few BCH tissues contained evidence of polyploidy or CNAs. Notably, however, several mutations associated with ESCC were also found in BCH samples, including those in *TP53, NOTCH1, CDKN2A, EP300,* and *MLL2.*[90] All these mutations were found in BCH, IEN, and ESCC tissues, so they might be early clonal events involved in development of ESCC. Independent studies also showed that *TP53* mutations persisted from IEN to invasive cancer.[91,92]

It is not clear how these mutations promote esophageal tumorigenesis. A model was proposed in which mutations in p53 and Krüppel-like factor 5 (KLF5, a zinc finger-containing transcription factor) contribute to transformation of esophageal squamous cells through de-regulation of the NOTCH1 pathway. In this model, wild-type p53 binds to the *NOTCH1* promoter to activate its expression in healthy esophageal cells. In dysplastic esophageal cells, with a *TP53* mutation, KLF5 replaces p53 to maintain *NOTCH1* transcription. Subsequent loss of KLF5 reduces expression of *NOTCH1*, leading to transformation of esophageal squamous cells.[89] However, this model is based on findings from cultured cells and xenograft tumors. Studies of genetically engineered mice that develop spontaneous esophageal cancer could help delineate further the genetic changes involved.

Compared with BCH, LGIEN and HGIEN contain more genomic and epigenomic alterations, including CNAs, gene mutations, loss of heterozygosity, and regions of promoter hypermethylation. Most of these alterations are present throughout the invasive tumor (Figure 1).[90] Gains of 3q, 5p, 7q, 8q, and 11q13 and losses of 3p and 9p were observed in esophageal tissues with mild dysplasia, so these might be early events. In comparison, gains of 16p, 17q, 19p, 19q, 20p, 20q, and 22q and losses of 4p, 4q, 5q, 8p, 18q, and 21q were detected in only invasive tumor tissues, and are therefore likely to be late alterations. In precancer lesions, amplification of 7p11.2 (locus of *EGFR*), 11q13.2–q13.3 (*CCND1*), 8q24.21 (*MYC*), and 3q26.32–q26.33 (*SOX2* and *PIK3CA*) and homozygous deletion of 9p21.3 (*CDKN2A*) are the most frequently detected aberrations.[10,90] These focal CNAs appear to be early driving events, consistent with findings from bioinformatic, multiregion genomic analyses.[73]

The number of somatic mutations detected in IENs is surprisingly comparable with that of ESCCs (4.56 and 3.55 mutations/Mb of DNA, respectively).[90] Dysplastic lesions share a mutation spectrum with their matched esophageal cancer. Each have a APOBEC signature, indicating increased deamination activity even before development of ESCC. Some driver genes were also mutated at similar frequencies in IEN compared with matched ESCC

samples, including *TP53, NOTCH1, CDKN2A, FAT1, PIK3CA, RB1, EP300,* and *MLL2*. In a mathematical assessment, a large fraction of these mutations was detected as fully clonal in precancerous lesions, so they might provide the cell with a proliferative or survival advantage. Other alterations detected in dysplasia include hypermethylation at promoters of tumor suppressor genes (*CDKN2A*, *CLDN3*, and *MT1G*)[93,94] and loss of heterozygosity of microsatellite markers.[95–98] Together, these findings indicate that even though IEN and ESCC are histologically distinct, they share many pathogenic genomic abnormalities. These might be used as biomarkers for early detection and risk stratification.

ESCC Signaling Pathways and Therapeutic Targets

Several signaling pathways are dysregulated in ESCCs via genomic and epigenomic aberrations. In many types of SCC cells, an antioxidative signaling pathway is activated by genomic alterations. In this pathway, NRF2 (encoded by *NFE2L2*) activates transcription of genes that control oxidative stress. KEAP1 interacts with CUL3 to target NRF2 for degradation through the ubiquitin-proteasome system. In ESCC samples, gain of function mutations and copy number gains activate NRF2, whereas loss-of-function mutations and copy number loss inhibit KEAP1 and CUL3. ATG7, a direct regulator of autophagy-mediated KEAP1 turnover,[99] is also deleted in a subset of ESCCs.[35] Together, these genomic changes increase activity of the antioxidative pathway in ESCCs, as well as other SCCs (such as HNSCC, LUSC, and skin SCC).

These findings provide evidence for loss of oxidative homeostasis during pathogenesis of squamous cell neoplasms. Increased activity of antioxidative signaling pathways contributes to resistance of cancer cells to chemotherapeutic agents, by reducing cell stress and inhibiting apoptosis (reviewed by Sporn and Liby[100]). The NRF2 pathway has become a target for development of antineoplastic agents. Several natural compounds (trig-onelline,[101] apigenin,[102] and brusatol[103]) reduce NRF2 activity through various mechanisms (Table 4). Preclinical studies of these inhibitors demonstrated their antitumor properties and synergistic effect with chemotherapeutic agents.

Squamous cell differentiation is also disrupted in SCCs. Squamous cell differentiation is controlled by lineage-specific transcription factors including NOTCH1, SOX2, TP63, and ZNF750. *SOX2* and *TP63* are often coamplified,[22] whereas *NOTCH1*, *NOTCH3*, and *ZNF750* are frequently mutated in ESCCs and other SCCs. These transcription factors interact and function together in squamous cells (eg, NOTCH1 interacts with TP63,[104] SOX2 interacts with TP63,[105] and TP63 regulates ZNF750[106,107]). Therefore, these genomic changes converge to dysregulate the differentiation program of squamous cells, which can have profound effects on the biology of ESCC.

ESCCs contain other genomic and epigenomic defects associated with hallmarks of cancer. Some of these dysregulate the cell cycle, such as mutations in *TP53* and *CDKN2A* (as well as deletion and promoter hypermethylation of this gene) and amplifications of *CCND1* and *CDK6*. As many as 98% of ESCC samples, from different cohorts, contain at least 1 of these aberrations,[12,33–35] underscoring cell-cycle deregulation as a common characteristic in ESCC patients. Palbociclib, a specific inhibitor of CDK4 and CDK6, was approved in 2015

by the Food and Drug Administration for treatment of estrogen receptor–positive breast tumors. Palbociclib and abemaciclib (another small-molecule inhibitor of CDK4 and CDK6) are currently being tested in phase 2 trials of patients with stage 4 LUSC (NCT02785939 and NCT02450539). Because LUSC has many genomic aberrations similar to ESCC, positive results from these studies could lead to trials in patients with ESCC.

Many mutations detected in ESCCs affect receptor tyrosine kinase signaling pathways. ESCCs contain amplifications in *EGFR*, *FGFR1*, and *KRAS* along with activating mutations in *PIK3CA*. In many ESCCs, *PTEN* is deleted or contains loss-of-function mutations. Some ESCCs have mutations in *ERBB2, ERBB4, MET*, and *MTOR* (Table 4). Interestingly, even though these genes are not mutated at high prevalences in ESCCs, their products (ERBB2, MET, and MTOR) are frequently overexpressed, indicating epigenetic, posttranscriptional, or posttranslational alterations (Table 4). Importantly, many dysregulated kinases are inhibited by drugs that have already been approved by the Food and Drug Administration or by agents in late stages of development for other types of cancer, underscoring the potential therapeutic merit of this pathway in ESCC.

Genomic lesions that activate Wnt signaling to β-catenin have been reported in ESCCs, including disruptions in *FAT1* or *AJUBA* (their products control β-catenin turnover)[108,109] and amplification of *YAP1* (its product promotes the transcription activity of β-catenin).[110] There are at least 26 different chemicals that affect Wnt signaling to β-catenin[111]; some of these are being studied in clinical trials, including a phase 2 trial of patients with metastatic HNSCC (NCT02649530). Results from the trial will be valuable for designing strategies to inhibit this pathway in ESCCs. Other pathways that are dysregulated in ESCCs include the nuclear exportin process (by mutations in *XPO1*) and homologous recombination pathway (by mutations in *BRCA1* and *BRCA2*).

Future Directions

Our understanding of the pathogenesis of ESCC has increased substantially, at an unparalleled speed, with developments in genomic, epigenomic, and proteomic technologies. However, many investigations into the genomic and epigenomic features of ESCCs are still in their infancy. For example, we still require comprehensive analyses of ESCC genomes, through large-scale WGS profiling. This approach would allow for discovery of functional noncoding variants, chromosome structural disorders, and mutation signatures of high confidence. Little is known about changes that occur in DNA methylome during ESCC development; understanding these will require whole-genome bisulfite sequence analyses, at single-base levels of resolution. These data also can be used to determine inter- and intratumor heterogeneity patterns of methylation.[112] Genome-wide histone modifications and insulated chromatin domains also await characterization in primary ESCC samples. These data would identify lineage-, cancer-, and subtype-specific cis-regulatory elements. It is also important to identify trans-regulatory factors and cofactors that interact with these functional DNA elements. Establishing a catalog of these cis-regulatory elements and trans-regulatory factors would greatly increase our understanding of the molecular factors that regulate the ESCC transcriptome.

Relating chromatin modifications to DNA methylome could uncover methylation-mediated dysregulation of chromatin functions. For example, hypomethylation activates enhancers[113] as well as super-enhancers[114] and disrupts insulated topological domains.[60] All of these mechanisms contribute to carcinogenesis via deregulation of gene expression programs.

Survival times of patients with ESCC could be greatly increased with earlier detection and diagnosis, which allow for earlier treatment. Biomarkers for early detection are not yet available despite efforts by researchers. High-resolution genomic and epigenomic profile and bio-informatic analyses of precancerous lesions and their matched ESCCs could provide insights into processes of tumor development, and lead to identification of bio-markers for cancer detection. Together with advanced imaging and endoscopic techniques, these tools will improve risk assessment of patients with esophageal dysplasia and early detection of ESCC.

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Abbreviations used in this paper

ADAR	adenosine deaminases acting on RNA
APOBEC	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like
BCH	basal cell hyperplasia
CIS	carcinoma in situ
CNA	copy number alteration
ESCC	esophageal squamous cell carcinoma
FGFR	fibroblast growth factor receptor
HGIEN	high-grade IEN
HNSCC	head and neck squamous cell carcinoma
IEN	intraepithelial neoplasia

KLF5	Krüppel-like factor 5
LGIEN	low-grade IEN
LINE-1	long interspersed nuclear element-1
LUSC	squamous cell carcinoma of the lung
NRF2	nuclear factor, erythroid 2 like 2
SMG	significantly mutated gene
WES	whole-exome sequencing.

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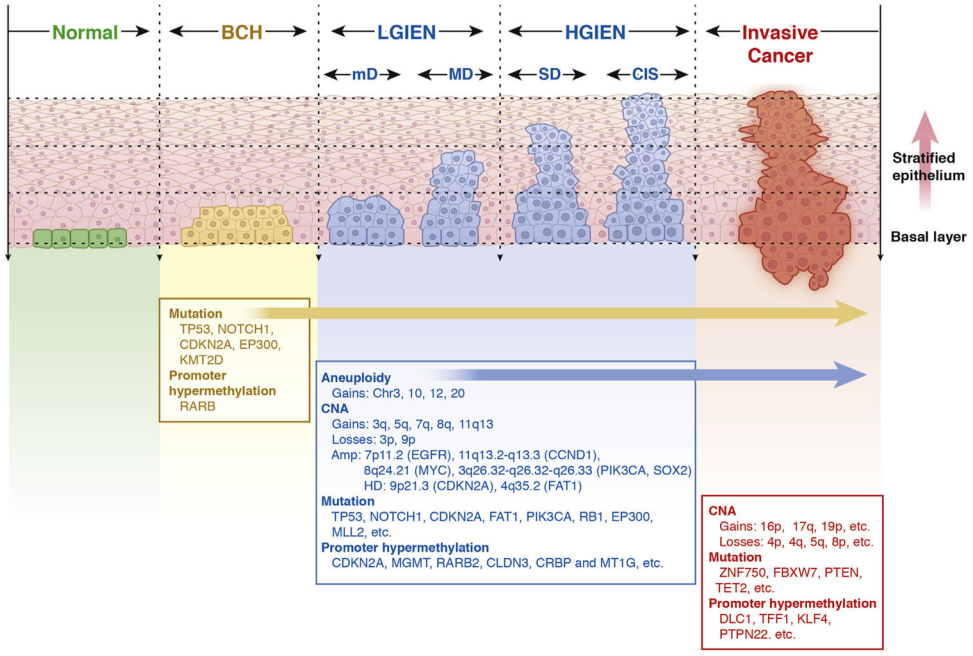

Figure 1.
Molecular Progression of Esophageal Squamous Precancerous Lesions to ESCC.

Table 1

Recurrent Focal CNAs in ESCCs

		Studies of ESCC genomic changes (Number of cases)								
Alteration	**Cytoband**	**Hu et al. SNP array (n = 30)**	**Bass et al. SNP array (n = 29)**	**Song et al. array-CGH (n = 123)**	**Lin et al. array-CGH (n = 59) SNP array (n = 22)**	**Gao et al. WES data (n = 81)**	**The Cancer Genome Atlas Research Network SNP array (n = 90)**	**Positive case no.**	**Overall frequancy (n = 434)**	**Driver gene in peak**
High-level amplification	11q13.2–q13.3	14	14	49	37	42	49	205	47.2%	*CCND1, FGF3/4/19, CTTN, CPT1A, ANO1, MIR548K*
	3q26.32–q26.33	1	2	27	2	3	17	52	12.0%	*PIK3CA, SOX2*
	7p11.2	1	4	12	5	6	10	38	8.8%	*EGFR*
	3q28	0	0	11	2	7	12	32	7.4%	*TP63*
	8q24.21	4	0	12	5	2	5	28	6.5%	*MYC*
	14q13.3	1	1	7	3	4	4	20	4.6%	*NKX2-1*
	8p11.23	2	2	3	6	1	5	19	4.4%	*FGFR1*
	12q15	0	0	7	6	1	4	18	4.1%	*MDM2*
	12p12.1	2	1	2	3	2	6	16	3.7%	*KRAS*
HD	9p21.3	9	15	12	13	14	52	115	26.5%	*CDKN2A/B*
	2q22.1–q22.2	0	3	1	0	0	17	21	4.8%	*LRP1B*
	3p14.2	1	0	1	0	0	14	16	3.7%	*FHIT*
	9p24.1	0	2	1	1	0	7	11	2.5%	*PTPRD*
	5q12.1	0	1	1	1	0	5	8	1.8%	*PDE4D*

A-CGH, array-CGH; HD, homozygous deletion; SNP-A, SNP-array; TCGA, The Cancer Genome Atlas.

Table 2

Significantly Mutated Genes in ESCC

Gene	Song et al. (n = 88)[8]	Lin et al. (n = 139)[12]	Gao et al. (n = 113)[33]	Sawada et al. (n = 144)[34]	TCGA (n = 97)[35]	Occurring cohorts	Prevalence (%)	Additional Aberrations	Validation in ESCC cells
TP53	1	1	1	1	1	5	80.5		Tumor-suppressor
NOTCH1	1	1	1	1	1	5	13.2		Tumor-suppressor
NFE2L2	1	1	1	1	1	5	9.3	Amplification	Oncogene[115]
KMT2D		1	1	1	1	4	15.2		
CDKN2A	1	1	1	1		4	5.8	Deletion, hypermethylation	Tumor-suppressor
ZNF750		1		1	1	3	9.0	Deletion	Tumor-suppressor[6,12]
PIK3CA	1	1		1		3	8.2	Amplification	Oncogene
RB1	1	1	1			3	4.8	Deletion	Tumor-suppressor
FAT1		1		1		2	8.8	Deletion	Tumor-suppressor[12]
EP300		1		1		2	6.5		Tumor-suppressor[33]
FBXW7			1	1		2	4.2	Deletion	Tumor-suppressor[116]
TGFBR2				1	1	2	3.2		
AJUBA			1	1		2	2.8		Tumor-suppressor[6]
CREBBP				1		1	5.5	Loss of heterozygosity[117]	
FAT2		1				1	5.0		Tumor-suppressor[12]
NOTCH3				1		1	4.8		Tumor-suppressor[118]
PTCH1			1			1	4.5		
KDM6A		1				1	4.2	Deletion	
FAM135B	1					1	4.0		Oncogene[8]
TET2				1		1	3.2		Tumor-suppressor[34]
PTEN		1				1	2.2	Deletion	Tumor-suppressor
ADAM29	1					1	1.3		

NOTE. A total of 22 SMGs and their mutational prevalence are summarized from 5 sequencing studies.[8,12,33–35] The number of cohorts containing these SMGs are indicated by the heatmap. Additional genomic and epigenomic aberrations are also summarized.

Table 3

Epigenomic Aberrations and Their Target Genes in ESCC

Epigenomic regulation		Coding or noncoding genes	Cancer-associated alterations
DNA methylation		Coding genes: TFF1[48], Bin1[119], NID2[120], PTPN22[121], PRSS8[122], KLF4[123], HIC1[124], RASSF2[45], RASSF1A[125], RASSF5A[126], PAR4[127], FBXO32[128], IGFBP7[129], SPINT2[130], ADAMTS8[131], DIRAS1[132], Rab25[133], PTK6[134], CDH1[135], UPK1A[136], HIN-1[137], DCC[138], PLCD1[139], CRABP1[140], LRP1B[26], RARβ[141], TSLC1[142,143], CDKN2A/B[42,43], DLC1[45], FHIT[144]	Promoter hypermethylation with decreased transcription
		Non-coding RNAs: miR-21833, miR-12634, nc88635, miR-34a36 miR-37537	Promoter hypermethylation with decreased transcription
		NGALR[145], LINE-1[53–56]	Hypomethylation
Chromatin modification	Histone writers	EP300, CREBBP, MLL, MLL2, MLL3, NSD1, SETD2, JARID2	Somatic mutations
		EZH2[61], G9a[62]	Increased transcription
	Histone erasers	KDM6A, TET2, NCOR2	Somatic mutations
		HDAC4[146]	Increased transcription
	Chromatin remodelers	ARID1A, ARID2, SMARCC2	Somatic mutations
RNA editing		ADAR1[71]	Gene amplification with increased transcription
		ADAR2[72]	Decreased transcription

Table 4

Signaling Pathways Altered in ESCCs and Targeting Agents

Signaling pathway	Gene	Copy number alteration, %	Somatic mutation, %	Protein alteration, %	Targeting agents
RTKs and their downstream mediators	*EGFR*	18	2	37[147]	cetuximab, erlotinib, gefitinib
	ERBB2	3		37[148]	trastuzumab, lapatinib, neratinib
	ERBB4		3		pelitinib, BMS-599626
	KRAS	7			
	FGFR1	12		17[33]	dovitinib, BGJ398, AZD4547
	MET	4		43[149]	ARQ197, MetMab
	PIK3CA		8	57[147]	BEZ235, BKM120, XL147
	PTEN	3	2	25[149]	
	MTOR		2	25[150]	temsirolimus, everolimus, ridaforolimus
Oxidative stress	*NFE2L2*	3	9		trigonelline, apigenin, brusatol
	KEAP1		3		
	CUL3	2	2		
	ATG7	6			
Squamous cell differentiation	*SOX2*	39		53[151]	
	TP63	44		96[152]	
	NOTCH1		13		
	ZNF750	4	9	75[33]	
Cell cycle regulation	*TP53*		80	43[147]	Ad-p53, Ad-p53-dendritic cells, CDB3
	CDKN2A	66	6	90[43]	palbociclib, abemaciclib
	RB1	8	5		
	CCND1	57		58[153]	
	CDK6	16			
WNT-β-catenin	*FAT1*	7	9	33[33]	WNT974, ICG-001, PFK115–584
	AJUBA		3		
	YAP1	5		27	
Nuclear exportin	*XPO1*	3	1	48[33]	leptomycin B, KPT-SINE, KOS-2464
Homologous recombination	*BRCA1*		2		iniparib, BSI-201, olaparib
	BRCA2		3		

Signaling pathway	Gene	Copy number alteration, %	Somatic mutation, %	Protein alteration, %	Targeting agents
Gene amplification					
Gene deletion					
Gene mutation					
Promoter hypermethylation					
Protein up-regulation					
Protein down-regulation					

NOTE. Due to space limits, only representative targeting agents are shown.