Cellular and Molecular Biology of Esophageal Cancer

2

Alfred K. Lam

Introduction

Histological Differences

Esophageal cancers comprise cancers of different histological types of diverse cellular and molecular bases [1, 2]. The two major histological types of esophageal cancers are squamous cell carcinoma and adenocarcinoma. It is important to note that there are histological variants of both squamous cell carcinoma and adenocarcinoma, such as basaloid squamous cell carcinoma, spindle cell carcinoma, mucoepidermoid carcinoma, and adenosquamous carcinoma [3–6]. In addition, neuroendocrine neoplasms such as small cell carcinoma of the esophagus account for approximately 1% of primary esophageal carcinoma [7]. All these carcinomas have distinct clinicopathological features. Limited studies have revealed that the cellular and molecular biology of these uncommon types of esophageal carcinomas is different from those of esophageal squamous cell carcinoma or adenocarcinoma [4, 8, 9].

The current understanding of the cellular and molecular biology of esophageal cancers focuses on esophageal squamous cell carcinoma and esophageal adenocarcinoma. The difference in prevalence of these two major histological types in different geographic regions is likely due to the complex interactions of genetic and environmental factors. In general, esophageal squamous cell carcinoma predominates in areas with high incidence of esophageal cancer, whereas esophageal adenocarcinoma is more common in areas with low incidence of esophageal cancer. In addition, the genetic mechanisms of esophageal squamous cell carcinoma are complex with multiple genetic factors proposed [2]. On the other hand, most esophageal

A. K. Lam (✉)
School of Medicine, Griffith University and Pathology Queensland, Gold Coast University Hospital, Gold Coast, Australia
e-mail: a.lam@griffith.edu.au

N. F. Saba, B. F. El-Rayes (eds.), *Esophageal Cancer*,
https://doi.org/10.1007/978-3-030-29832-6_2

adenocarcinomas show genetic changes of the progression of lesions related to acid reflux. The histological progression from reflux esophagitis to Barrett's metaplasia to dysplasia to adenocarcinoma is well known.

Applications of Molecular and Cellular Biology

Esophageal cancer is one of the leading causes of cancer death worldwide despite recent improvements in surgical and adjuvant therapies. Better understanding of the cellular and molecular biology of these cancers will allow us to apply this knowledge to clinical management, thereby increasing the quality of life of patients with esophageal cancer. Thus, the study of cellular and molecular biology of esophageal cancer serves the following purposes: (1) to establish the presence or absence of an infectious cofactor, (2) to understand the genetic mechanisms of disease, (3) to provide prognostic information, and (4) to predict response to medical therapies and new modalities of treatment. In performing research and interpreting and applying knowledge in this area, it is important to bear in mind the histological differences between esophageal cancers.

Establishment of an Infectious Cofactor

For esophageal adenocarcinoma, gastroesophageal reflux and the resulting Barrett's esophagus (intestinal metaplasia) are the most important risk factors [1]. Obesity, tobacco use, drugs, and dietary factors also play roles as risk factors [10]. Besides these, the role of infection in the development of esophageal cancer has long been suspected, in particular the role of human papillomavirus (HPV).

Human Papillomavirus

In esophageal cancers, the main infectious cofactor under intensive study is HPV. HPV is a non-enveloped double-stranded DNA virus that can infect the basal cells of the skin or mucosa. The majority of patients with HPV infections are asymptomatic. After the infection, approximately 10% of patients may have persistent infection, which may lead to cancer [11]. In squamous cell carcinomas of the upper aerodigestive tract, in particular in the oropharynx, identification of the presence of HPV in the carcinomas is of important value [12]. In these sites, patients with HPV-positive cancers have better prognosis when compared to patients with HPV-negative cancers. The detection of HPV in oropharyngeal squamous cell carcinomas also predicts better response to radiotherapy. The detection of HPV in clinical settings is indirectly achieved by the identification of expression of p16 protein by immunohistochemistry (IHC) [13].

The esophagus is distal to the oropharynx and histologically lined by stratified squamous epithelium as in the oropharynx. Studies to investigate HPV in esophageal

squamous cell carcinomas have been underway for 30 years [14, 15]. Thus, there is considerable data on the role of HPV infection in the development of esophageal cancer. The majority of studies were in esophageal squamous cell carcinoma.

Pooled analysis of five studies (in the years 2006–2013) from the literature revealed that HPV prevalence in esophageal adenocarcinoma was 35.0% (range, 1–90%) and HPV-16 prevalence was 11.4% [16]. Due to the limited number of studies on esophageal adenocarcinoma, no detailed analysis of the impact was available. Nevertheless, the hypothesis is that progressive acid damage to the esophagus increases the likelihood of mucosal breaks and allows the virus to enter the basal layer of the transformation zone. Recently, transcriptionally active HPV was noted to be strongly associated with Barrett's dysplasia and esophageal adenocarcinoma, suggesting a potential role of HPV in esophageal carcinogenesis. The involvement of HPV is reported to be via wild-type p53 and aberrations of the retinoblastoma protein pathway [17]. On the other hand, Antonsoon and colleagues in 2016 showed no evidence of HPV DNA in a large cohort (n = 233) of histologically confirmed archived esophageal adenocarcinomas [18]. Thus, HPV alone is unlikely to cause esophageal adenocarcinoma.

In esophageal squamous cell carcinoma, summarized HPV prevalence from both early and recent meta-analysis was 22% [16]. In general, HPV prevalence was higher in studies conducted in Asian countries and was much lower in studies conducted in Western countries such as in Europe and America [2]. Stratified analysis by localization of cancer showed that esophageal squamous cell carcinoma was only slightly higher in the cervical portion but not significantly higher than the middle or lower portion of the esophagus [19].

With respect to HPV DNA detection in meta-analysis, the prevalence of esophageal squamous cell carcinoma detected by type-specific primer PCR method (30.4%) was significantly higher than that by broad-spectrum primers (20.8%) [16]. Limited studies have employed the IHC method to detect p16 protein to study HPV infection in esophageal carcinoma. Nevertheless, the current data using p16 detection in esophageal squamous cell carcinoma did not reflect the HPV status in the cancer [20]. Detection of HPV DNA is thus the preferred means of studying HPV in esophageal carcinoma.

Human papillomaviruses are a group of more than 100 subtypes of viruses [11]. Slightly more than 30 subtypes are oncogenic in humans and are defined as high risk and low risk for cancers [21]. From pooled data, HPV-16 was the most frequently observed subtype with a summarized prevalence of 11.4% [2, 16]. The other six most frequent individual HPV subtypes identified in esophageal squamous cell carcinoma, in order of decreasing prevalence, were HPV-18 (2.9%), HPV-6 (2.1%), HPV-11(2.0%), HPV-52 (1.1%), HPV-33 (0.8%), and HPV-31 (0.6%). Apart from HPV-6 (low-risk type), all the detected types belong to high-risk carcinogenic HPV types. HPV-16 can induce cancer stem-like cell phenotypes in esophageal squamous cell carcinoma through the activation of the p13K/AKT signaling pathway [22].

Overall, HPV infection was associated with an increased risk of esophageal squamous cell carcinoma. However, the association was not as strong as that for

oropharyngeal squamous cell carcinoma or cervical squamous cell carcinoma. The impact on survival of patients with esophageal squamous cell carcinoma has not been clearly determined. Patients with HPV-positive esophageal squamous cell carcinoma had better response to chemoradiation [23, 24]. Wang and colleagues also reported better 3-year survival in patients with HPV-positive cancers [24]. On the other hand, de Costa and colleagues showed no predictive values of HPV, p16, and p53 status on the survival of patients with esophageal squamous cell carcinoma in a recent multivariate analysis [25]. At this stage, routine evaluation of HPV or p16 status is not required in the management of esophageal cancer.

The importance of studying the pathogenesis of HPV in cancers also stems from the availability of effective vaccines against HPV in the market. Prophylactic HPV vaccine is now in its second generation [26]. The vaccine is useful to prevent premalignant genital and anal lesions arising from infection with HPV when given to young females. Australia was the first country to offer complimentary HPV vaccines to boys and girls. The clinical impact of the vaccination program is already visible in the population. Although there is no data from clinical trials regarding the efficacy of the vaccines for HPV-related cancers outside the genital tract, it is likely that universal vaccination could affect the prevalence of HPV-related esophageal cancers in the future.

Epstein-Barr Virus

The detection rates of Epstein-Barr virus (EBV) in esophageal cancer are variable and range from 0% to 35% [27–29]. This variability likely results from differences in racial, geographical, and detection methods used. It is worth noting that lymphocytes in the cancer stroma can harbor EBV, and thus detection of virus in esophageal cancer by PCR-based methods may show false-positive results [28]. On the other hand, in situ hybridization may provide false-negative results due to a higher rate of RNA degradation. Most studies have shown that EBV-associated esophageal cancer demonstrates similar morphologic findings to undifferentiated carcinoma of the nasopharynx, which is associated with EBV. At the current time, the identification of EBV in esophageal carcinoma has no clinical application.

Bacteria

Helicobacter pylori, previously known as *Campylobacter pylori*, is a Gram-negative microaerophilic spiral bacterium, which is the major cause of peptic ulcer disease and a recognized cause of gastric carcinoma. Some strains of *H. pylori* may protect patients from gastroesophageal reflux disease and esophageal adenocarcinoma [27, 29]. This effect may result from the bacterium decreasing acid production through the production of cytokines [29]. It is worth noting that the decreased prevalence of *H. pylori* worldwide because of antibiotics use parallels the increased prevalence of esophageal adenocarcinoma [29]. Overall, there is no consensus on the role of *H. pylori* in esophageal adenocarcinoma, with substantial differences between the

results of Asian and Western studies. Metagenomics studies have identified many other types of bacteria in the esophagus [27, 30]. Metagenomics is the study of microbiota in their natural habitat using next-generation sequencing through a PCR-based analysis of bacterial 16S rRNA genes. Two distinct clusters, a predominantly Gram-positive cluster (type I) and a predominately Gram-negative cluster (type II), were noted. The type II cluster may stimulate expression of different proteins and genes leading to reflux and trigger the process of adenocarcinoma formation.

Understanding Genetic Mechanisms

Genetic Profiles

Esophageal carcinomas are biologically aggressive cancers and thus their genetic profiles are complex. Oncogenes, tumor-suppressor genes, metastatic genes, apoptosis genes, proliferation-related factors, epigenetic factors, and proteins related to metastases have roles in the pathogenesis of both esophageal squamous cell carcinoma and esophageal adenocarcinoma [2, 31]. In recent years, studies have suggested that many components of the *P13/AKT* (phosphatidylinositol 3-kinase/protein kinase B) pathway may be important in the pathogenesis of esophageal squamous cell carcinoma. The expressions of different markers such as E-cadherin, N-cadherin, p120, DNAJB6 (DnaJ homolog subfamily B member 6), and phosphorylated AKT play roles in progression of the cancer as well as predicting the prognosis of patients with the cancer [32–34]. Oncogenic proteins such as receptors for vascular endothelial growth factor (VEGF) and calpain 10 (CAPN10), which is regulated by gene amplified in esophageal cancer 1 (GAEC1), are related to the clinical progression of esophageal squamous cell carcinoma [35, 36]. In addition, epigenetic changes such as promoter methylation of nidogen-2 (NID2, a key component of the basement membrane) could suppress the epidermal growth factor receptor (EGFR)/AKT metastasis-related pathway and control cancer metastases [37]. In general, for both esophageal squamous and adenocarcinoma, p53 mutation is an important genetic change [38, 39].

DNA copy number alterations and methylation analysis could detect many of the genetic and epigenetic changes in esophageal carcinomas [40–43]. Studies from about 2000 onwards have used comparative genomic hybridization (CGH) and expression array to identify the differences in genetic profiles between esophageal cancer and noncancerous esophageal tissue [44–47]. Chromosomal regions with amplification may harbor oncogenes, and chromosomal regions with deletion may harbor tumor-suppressor genes. CGH can identify the whole profile of cytogenetic changes in an individual cancer. Using this approach, researchers have identified many new cancer-related genes in both esophageal squamous cell carcinoma and esophageal adenocarcinomas [48–54]. These provide more information regarding the carcinogenesis of esophageal cancers as well as defining gene candidates as prognostic markers and molecular targets for therapy.

The traditional method of detecting genetic mutations is by Sanger sequencing [55]. The introduction of next-generation sequencing in research and clinical

practice has led to the sequencing of many new genes and generated vast quantities of genetic data at a low cost [56, 57]. These recent technologies allow researchers to sequence DNA much more quickly and economically than the previously used Sanger sequencing and as such have revolutionized the study of genomics and molecular biology. The first commercially available next-generation sequencer was available in 2007, and many newer versions offer the ability to detect multiple genes in one experimental run using smaller size equipment (Fig. 2.1). Using these robust new sequencing platforms, whole exome sequencing and whole genome sequencing of patients with esophageal carcinoma are possible. In the literature, reports of whole exome sequencing have been noted mainly in esophageal squamous cell carcinoma and occasionally in esophageal adenocarcinoma [58–67]. Many novel mutations and genetic pathways have been detected which could help us to understand the pathogenesis of this group of cancers with complex genetic alterations (Table 2.1).

The International Cancer Genome Consortium (ICGC) coordinates a large number of research projects that have the common aim of comprehensively elucidating the genomic changes present in many cancers [68]. The preliminary meeting was in 2007 and the consortium launched a public notice in 2010. The primary goals of the ICGC are to generate comprehensive catalogues of genomic abnormalities (somatic mutations, abnormal expression of genes, epigenetic modifications). For esophageal cancer, the genomic study of esophageal squamous cell carcinoma was conducted by researchers in China, whereas the study of esophageal adenocarcinoma was performed by researchers in the United Kingdom.

Whole genome sequencing data for esophageal cancer began to appear in the literature in 2013 [69–83]. A large volume of information is available for the two major histological subtypes of esophageal cancer, which provides substantial resources for future research directions for the better management of patients with esophageal carcinoma (Table 2.1). The information includes (1) the first report of many novel driver gene mutations, (2) the relevant frequencies of key mutations in esophageal carcinomas, (3) the identification of predominant mutation pathways in esophageal cancers, (4) mutational signatures related to risk factors and (5) progression of the cancer as well as changes related to adjuvant chemotherapy. It is worth noting that as predicted from the biological aggressiveness of esophageal cancer, the genomic changes obtained are very complex. It will take time for research into

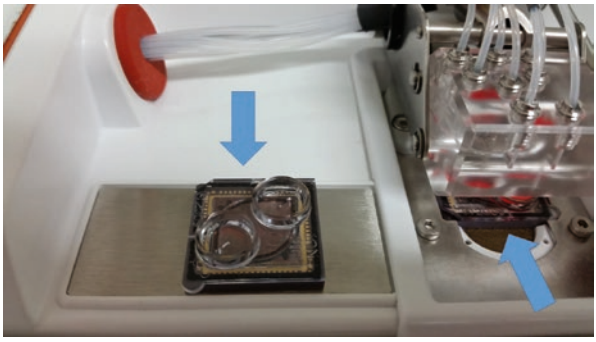

Fig. 2.1 Use of next-generation sequencer to study esophageal carcinoma. A chip (arrow) in which DNA to be sequenced is loaded. On the right side, the chip (arrow) is in the grounding plate on the benchtop sequencer

Table 2.1 Whole exome and whole genome sequencing results in esophageal carcinoma

Author/year/place	Samples	Findings
Whole exome sequencing in esophageal carcinoma		
Lin/2014/China	139 ESCC	Novel mutated genes, RTK-MAPK-PI3K pathways, cell cycle, and epigenetic regulation are frequently dysregulated
Wang/2015/China	9 ESCC and matched blood samples	Importance of deletion of 9p21.3 covering *CDKN2A/2B*, amplification of 11q13.3 covering *CCND1*, and *p53* mutation
Stachler/2015/USA	30 EAC and Barrett's esophagus	Importance of p53 in the progression
Raiendra/2016/Australia	EAC (4 HPV-positive and 78 HPV-negative)	Distinct genomic differences between HPV-positive and HPV-negative EAC
Findlay/2016/UK	30 EAC before and after neoadjuvant chemotherapy	Changes in driver mutations and acquire new mutations after chemotherapy
Liu/2016/Africa	59 ESCC	Mutational signature analysis revealed common signatures associated with aging, cytidine deaminase activity (APOBEC), and a third signature of unknown origin
Hao/2016/China	13 ESCC	Evidence of spatial intra-tumor heterogeneity with multiple mutations
Chen/2017/China	45 ESCC with matched dysplasia	Mutations in p53 and gains in 3q are early alterations in ESCC development
Forouzanfar/2017/Iran	9 familial ESCC	Identify Notch signaling pathway in ESCC pathogenesis
Dai/2017/Hong Kong	41 ESCC with 15 matched lymph nodes with ESCC	Critical roles of ZNF750 mutations, TP53 putative GOF mutations, and nucleosome disorganization in ESCC metastasis
Whole genome sequencing in esophageal carcinoma		
Dulak/2013/UK	15 EAC	Novel genes (include chromatin-modifying factors and candidate contributors *SPG20*, *TLR4*, *ELMO1*, and *DOCK2*) identified as well as the potential activation of the *RAC1* pathway
Song/2014/China	17 ESCC	Frequent mutations in well-known tumor-associated genes (*p53*, *RB1*, *CDKN2A, PIK3CA*, *NOTCH1*, *NFE2L2*), and two novel genes (*ADAM29* and *FAM135B*) as well as in histone regulator genes
Nones/2014/Australia	22 EAC	Oncogene amplification through chromothripsis-derived double-minute chromosome formation (*MYC* and *MDM2*) or breakage-fusion-bridge (*KRAS*, *MDM2*, and *RFC3*). Telomere shortening is more prominent in EACs bearing localized complex rearrangements. Mutational signature analysis also confirms that extreme genomic instability in EAC can be driven by somatic BRCA2 mutations
Weaver/2014/UK	12 EAC and Barrett' esophagus	The majority of recurrently mutated genes in EAC were mutated in non-dysplastic Barrett's esophagus. Only *p53* and *SMAD4* mutations occurred in a stage-specific manner, confined to high-grade dysplasia and EAC

(continued)

Table 2.1 (continued)

Author/year/place	Samples	Findings
Paterson/2015/UK	22 EAC and matched normal tissue/blood	Somatic mobile elements insertions are abundant in EAC
Ross-Innes/2015/UK	23 pairs of EAC and Barrett's esophagus	(i) Barrett's esophagus is polyclonal and highly mutated even in the absence of dysplasia; (ii) when cancer develops, copy number increases and heterogeneity persists such that the spectrum of mutations often shows little overlap between EAC and adjacent Barrett's esophagus; and (iii) despite differences in specific coding mutations, the mutational context suggests a common causative insult underlying these two conditions
Zhang/2015/China	104 ESC and previous reports	Cytidine deaminase activity (APOBEC)-mediated mutational signature, high activity of hedgehog signaling, and the PI3K pathway
Qin/2016/China	10 ESCC	Identify mutations in *VANGL1* as well as in three coding genes (*SHANK2*, *MYBL2*, *FADD*) and two noncoding genes (*miR-4707-5p*, *PCAT1*)
Sawada/2016/Japan	144 ESCC	Patients were assigned to three groups, which are associated with environmental (drinking and smoking) and genetic (polymorphisms in *ALDH2* and *CYP2A6*) factors. Many tumors contained mutations in genes that regulate the cell cycle, epigenetic processes, and the *NOTCH*, *WNT*, and *receptor-tyrosine kinase-phosphoinositide 3-kinase* signaling pathways
Secrier/2016/UK	129 EAC	Mutational signatures showed three distinct molecular subtypes with potential therapeutic implication: (i) enrichment for BRCA signature with prevalent defects in the homologous recombination pathway, (ii) dominant T > G mutational pattern associated with a high mutational load and neoantigen burden, and (iii) C > A/T mutational pattern with evidence of an aging imprint
Cheng/2016/China	31 ESCC	Molecular defects such as chromothripsis and breakage-fusion-bridge are important in malignant transformation of ESCCs and demonstrate diverse models of somatic variation-derived target genes in ESCCs
Cheng/2016/China	A portion of 104 ESCC (stage I or II)	*FAM84B* and the *NOTCH* pathway are involved in the progression of ESCC
Fels Elliott/2017/UK	171 EAC	Toll-like receptor pathway genes are recurrently mutated
Noorani/2017/UK	10 EAC matched pre- and post-chemotherapy	The genomic landscape of pre- and post-chemotherapy is similar for EAC
Liu/2017/China	70 ESCC and squamous dysplasia	Squamous dysplasia and ESCCs each had similar mutations and markers of genomic instability, including apolipoprotein B messenger RNA editing enzyme, catalytic polypeptide-like

ESCC esophageal squamous cell carcinoma, EAC esophageal adenocarcinoma, HPV human papilloma virus

the functional aspects of these genomic changes to be applied to the clinical management of patients with this group of cancers.

MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are a class of small, well-conserved, non-coding RNAs that regulate the translation of RNAs. Many studies have shown that miRNAs have important biological and pathological functions in many cancer types [84–96]. miRNAs affect a variety of biological processes in the body as well as act as oncogenes, tumor-suppressor genes, or regulators of cancer stem cells. Due to their small size, there are established means of miRNA detection methods (traditional and new) in serum, cell lines, and human tissues in esophageal carcinoma [97, 98].

In esophageal adenocarcinomas, expression levels of different sets of miRNAs are altered during the development of adenocarcinoma from Barrett's esophagus. In different studies, miRNAs such as miRNA-192, miRNA-196, and miRNA-21 were frequently upregulated, whereas miRNA-203, miRNA-205, and miR-let-7 were commonly downregulated during the development from Barrett's esophagus to esophageal adenocarcinoma [99]. In addition, changes in the expression of miRNAs are associated with the prediction of metastasis, prognosis, and response to chemoradiation in patients with esophageal adenocarcinoma. Similarly, many miRNAs are involved in the pathogenesis of esophageal squamous cell carcinoma. miRNAs have oncogenic or suppressor roles as well as potential roles as diagnostic and prognostic markers in the cancer. Many more miRNAs have been identified in esophageal squamous cell carcinoma as the carcinoma has a more complex carcinogenesis than esophageal adenocarcinoma [100–102].

Experimental studies to manipulate miRNAs in cancer cell lines may provide new strategies for cancer therapeutics. However, further studies, such as how to deliver miRNAs specifically to cancer tissues, are required in order to be able to apply miRNAs for clinical use.

Cancer Stem Cells

Cancer stem cells (CSCs) are a subgroup of cancer cells with properties resembling the critical properties of embryonic stem cells such as self-renewal and maintenance of stemness [103–106]. Only cancer stem cells have tumor-initiating properties. CSCs are responsible for initiation, progression, metastases, and recurrence in cancer. They play an important role in the resistance of cancer to adjuvant therapies and in cancer recurrence via their activation of different signaling pathways such as Notch, Wnt/β-catenin, TGF-β, hedgehog, PI3K/AKT/mTOR, and JAK/STAT pathways [105, 106]. In addition, epithelial-mesenchymal transition (EMT) may be involved in epithelial cell immortalization and enrichment of stemness. These immortal cells may regain their original properties via mesenchymal-epithelial transition (MET) and maintain epithelial stem cell properties [107].

Identification of cancer stem cells is important in cancer and is challenging. CSCs are most often identified by detecting the expression of their antigens in a

group of stem cells [108]. Many surface markers can be used to detect CSCs by directly targeting their specific antigens present in cells. In addition, multiple analytical methods and techniques including functional assays, cell sorting, filtration approaches, and xenotransplantation methods can identify CSCs.

In esophageal squamous cell carcinoma, markers such as CD44, ALDH, Pygo2, MAML1, Twist1, Musashi1, side population (SP), CD271, and CD90 can be used to identify CSCs in individual cancer masses. In addition, stem cell markers like ALDH1, HIWI, OCT3/4, ABCG2, SOX2, SALL4, BMI-1, NANOG, CD133, and podoplanin are associated with patient prognosis, pathological stage, cancer recurrence, and therapy resistance [109]. In esophageal adenocarcinoma, CSCs are responsible for intrinsic and acquired chemotherapy resistance, which is associated with EMT regulation [110]. As in esophageal squamous cell carcinoma, different methods including functional assays, cell sorting using various intracellular & cell surface markers and xenotransplantation techniques can identify and separate out CSCs. None of these methods alone can guarantee complete isolation of the CSC population. Thus, a combination of methods may be used to detect and isolate CSCs.

The development of specific markers and signaling molecules to target esophageal carcinoma CSCs and the validation of these stem cells might provide the basis for a revolutionary treatment approach for the elimination and/or differentiation of CSCs in esophageal cancer. Emerging therapeutic tools based on specific properties and functions of CSCs may improve clinical outcome of esophageal carcinomas. Therefore, innovative insight into the biology of cancer stem cells and therapies targeted to cancer stem cells will help to achieve effective management of esophageal cancers.

Prognostic Information

Predication of Progression

Aneuploidy (detected by FISH/flow cytometry), promoter hypermethylation, and cyclin A protein expression have been shown to correlate with the progression from Barrett's esophagus to esophageal adenocarcinoma [111, 112]. Despite these findings, there is generally a lack of large prospective studies to validate the use of these markers in clinical practice. The most likely candidate for clinical application is p53 protein overexpression as determined by IHC, which correlates with neoplastic progression to esophageal adenocarcinoma. It could be a useful adjunct to determine the grade of dysplasia in Barrett's esophagus. In addition, the results have been validated in some studies and the procedure used is simple.

The expression or identification of cellular and molecular markers can predict the survival of patients with esophageal adenocarcinoma [113, 114]. Some of the more commonly described markers are EGFR1 and 2, transforming growth factor (TGF α and β1), p53, Ki-67, cyclin-dependent kinase inhibitor 1 (p21), B-cell lymphoma 2 (Bcl-2), cyclooxygenase-2 (COX-2), nuclear factor-κB (NF-κB), VEGF, tissue inhibitor of metalloproteinase (TIMP), and microsatellite instability (MSI). At present, there is no routine testing for these markers, as researchers have not validated these markers adequately in prospective studies.

In esophageal squamous cell carcinoma, many molecular and cellular markers are associated with patient prognosis. Expression levels of p21, p53, cyclin D1, Ki-67, and E-cadherin provide some prognostic information [33, 34, 36, 115, 116]. However, this approach is not widely used.

Guidelines for Medical Therapies

Prediction of Response to Medical Therapies

Preoperative chemoradiation is a standard treatment for esophageal cancers. In patients who undergo neoadjuvant chemoradiation therapy, histological regression of the primary cancer, indicated by percentage of residual viable cells, is an important prognostic factor in addition to nodal status and gender [117].

It is thus important to have a means to predict the response to chemoradiation. The grade of esophageal squamous cell carcinoma could potentially predict the response to preoperative chemotherapy [118]. Many molecular makers have been studied [119–122]. p53 protein is expected to be a representative biomarker. The cell cycle markers CDC25B and 14-3-3sigma have potential as response biomarkers independent of the p53 status. The DNA repair markers, p53R2 or ERCC1, VEGF, and hedgehog signaling pathway factor Gli-1 also have potential as predictive biomarkers. However, further studies are required to validate the findings. In esophageal adenocarcinoma, expression of EGFR, VEGF, NF-κB, and cDNA microarray could act as predictive factors for preoperative chemoradiation.

It is important to be aware of the histological changes after preoperative chemoradiation [3]. In the current AJCC (American Joint Committee on Cancer) guidelines for staging of esophageal carcinoma, patients having preoperative chemoradiation have different guidelines for pathological staging than those patients without preoperative therapy [123].

Predictors for Targeted Therapy

Targeted therapy involves targeting a specific gene mutation in the cancer. In clinical settings, oncologists use targeted therapies to treat melanoma, breast cancer, and colorectal cancer with promising results [124–128]. Testing the cancer tissues for molecular markers is useful to predict the response of the patients to these targeted therapies.

Of the potential targets trialed to date in esophageal cancer, EGFR (Her 1 and Her 2) and VEGF surface receptor antagonists have shown the most promising results [129–133]. For instance, overexpression of EGFR-1 is present in 1/3 to 2/3 of esophageal adenocarcinoma and squamous cell carcinoma tissues. Her 2 (also known as c-erbb2, CD340, and Neu) staining has been demonstrated in esophageal squamous cell carcinoma [134].

The most important advance in the molecular biology and oncology of esophageal adenocarcinoma at the gastroesophageal junction is the approval of anti-Her 2 therapy for the treatment of this cancer [135]. On October 20, 2010, the US Food and Drug Administration (FDA) granted approval for the use of trastuzumab (Herceptin), which

targets the Her 2 protein. Trastuzumab in combination with other chemotherapy is approved for the treatment of patients with Her 2 overexpressing metastatic esophageal adenocarcinoma at the gastroesophageal junction who have not received prior treatment for metastatic disease. The approval was based on the findings in many clinical trials that trastuzumab-based therapy offered a significant survival advantage for patients with Her 2 overexpressing locally advanced, recurrent, or metastatic gastric and gastroesophageal junctional adenocarcinomas when compared to conventional therapy alone. Approval of the use of trastuzumab by the US FDA was followed by authorities in other countries, e.g., the Therapeutic Goods Administration (TGA) in Australia.

Pathologists are required to determine the Her 2 status in biopsy or resection material from gastroesophageal junction tumors as well as metastatic sites. IHC and in situ hybridization (ISH) testing is used to assess the expression of Her 2. Precise testing of the Her 2 status is important, as Her 2 is the only biomarker established for patients with advanced esophageal adenocarcinoma of the gastroesophageal junction. Pathologists should ensure that biopsies or resection specimens used for testing are properly fixed and pathologically assessed [136]. In many clinical laboratories, the protocol adopted is a combination of testing of Her 2 by IHC and ISH. Her 2 staining is membranous in cancer cells and is scored as "negative, 1+, 2+, and 3+" depending on standard criteria. In many centers, for cases that are negative or "1+" by IHC, the patients are not considered candidates for anti-Her 2 therapy. In cases that are strongly positive (3+, as defined by strong and complete membranous reactivity), patients are candidates for anti-Her 2 therapy. Esophageal adenocarcinomas at the gastroesophageal junction that are equivocal (2+) in staining are typically tested by ISH to reach a decision regarding trastuzumab therapy.

Research Sources for Molecular and Cellular Studies in Esophageal Cancers

Tissue Studies

Human cancer can be studied at the tissue level when tumor tissue is surgically removed from the human body. These cancer tissues are without blood supply and degeneration will quickly occur. Cancer studies on these tissues can be performed in several ways. In clinical settings, cancer tissues are fixed in formalin and embedded in paraffin. Thin sections can be cut from the paraffin-embedded tissues, stained by hematoxylin and eosin, and examined by pathologists under light microscope. These sections are useful for various molecular studies. In fact, many esophageal cancer research findings derived from studies are performed on paraffin-embedded tissues. This approach has the benefit of providing superior morphological features for studying histological features as well as localization of biomarkers at the cellular level when compared with other methods (Fig. 2.2). It is worth noting that histological assessment is important before starting any further molecular research. It is important to confirm the presence of cancer and the proportion of cancer cells on histological examination of the tissue. Proper dissection and histological examination of cancer tissue provides information regarding histological type, grading, and

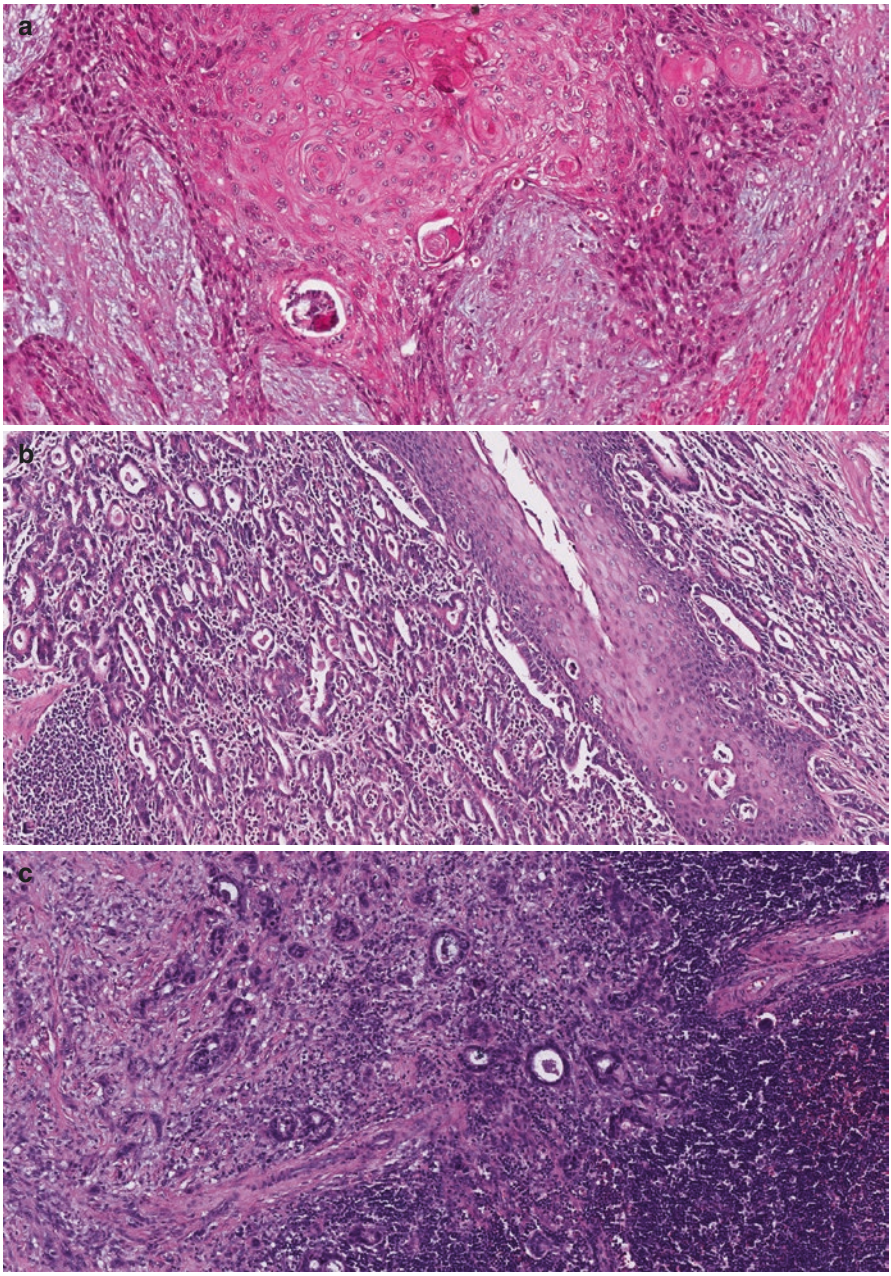

Fig. 2.2 Histological features of carcinomas from formalin-fixed and paraffin-embedded samples**.** (**a**) Well-differentiated squamous cell carcinoma. (**b**) Well-differentiated adenocarcinoma. (**c**) Lymph node with metastatic esophageal adenocarcinoma

pathological staging which are important parameters to determine the behavior of the cancer as well as the treatment options for esophageal carcinoma [3, 123, 137].

In recent years, the use of tissue microarray (TMA) has increased for testing molecular markers in large numbers of samples by either IHC or ISH (Fig. 2.3). The

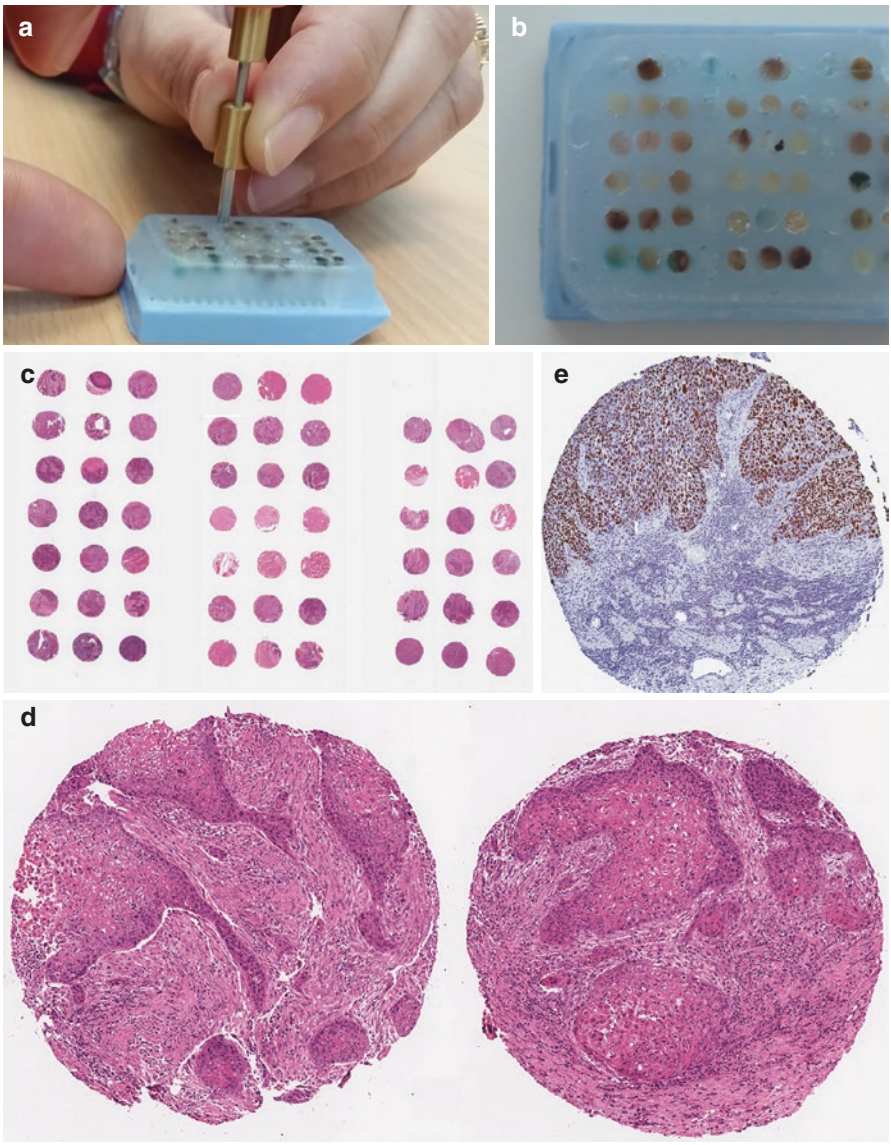

Fig. 2.3 Tissue microarray (TMA) of esophageal carcinoma. (**a**) Making tissue microarray block by manual technique. (**b**) A tissue microarray block with multiple tissue cores in the paraffin. (**c**) Section stained by hematoxylin and eosin taken from the tissue microarray block of esophageal squamous cell carcinoma. (**d**) Higher magnification of two of the cores of 3c. (**e**) The TMA section used to test a biological marker

testing of multiple samples in a block allows rapid screening of large numbers of patient samples and reduces the costs of reagents. The use of tissue in the form of TMA minimizes the amount of invaluable patient tissue used for research tests, making it available for essential clinical use. In the TMA technique, a hollow needle is used to remove tissue cores as small as 0.6 mm in diameter from regions of interest in each paraffin block. These tissue cores are then inserted in a recipient paraffin block in a precisely spaced array pattern [138]. The cores of tissues in the recipient block are from different patients. There are some drawbacks as cancer is heterogeneous, and small samples from a cancer may not represent the information that could be obtained by studying the whole tumor section. In addition, preparation and workup on the TMA blocks require greater technical expertise and time than conventional tissue blocks.

The drawback of working on paraffin-embedded tissues is that formalin irreversibly cross-links proteins via the amino groups, thus preserving the structural integrity of the cells to allow staining with dyes to analyze abnormalities in the tissue that indicate cancer. The effect of these cross-linking fixatives on the nucleic acids and proteins may impair molecular interactions. To overcome this drawback, snap-freezing in liquid nitrogen and storage at −80 °C is used to collect esophageal cancer tissues for use in research. The snap-freezing approach provides tissues that are superior in quality for molecular studies, for instance, whole genome or whole exome studies in esophageal carcinomas; however, the morphological features are inferior to those obtained using paraffin-embedded sections (Fig. 2.4).

The staining of histological sections will fade over time. In addition, storage of large amounts of histological sections is difficult. Whole-slide imaging allows scanning and storage of the histological slides in digital files [139]. This also allows long-term storage of research data as well as computerized analysis of histological parameters (Fig. 2.5). Researchers can share information more easily using digitalized slides.

Blood samples are also important research materials for patients with esophageal carcinomas. Blood can be used to analyze circulating DNA, miRNA, or CTCs in esophageal carcinoma patients [140, 141].

Cancer Cell Lines

It is worth noting that research with removed cancer tissue cannot provide functional dynamic studies of esophageal cancers. For functional studies in esophageal cancer, studies are often performed in cancer cell lines derived from tissues obtained freshly from surgery. Several molecular approaches are used to block the genetic changes in the cancer [142]. For instance, RNA interference (RNAi) is a normal physiological mechanism in which a short effector antisense RNA molecule regulates target gene expression. RNAi can silence a particular gene of interest in a sequence-specific manner and is used to target various molecular pathways in esophageal carcinoma by designing RNAi specific for key pathogenic genes. Several RNAi-based strategies are being explored to develop therapeutics against

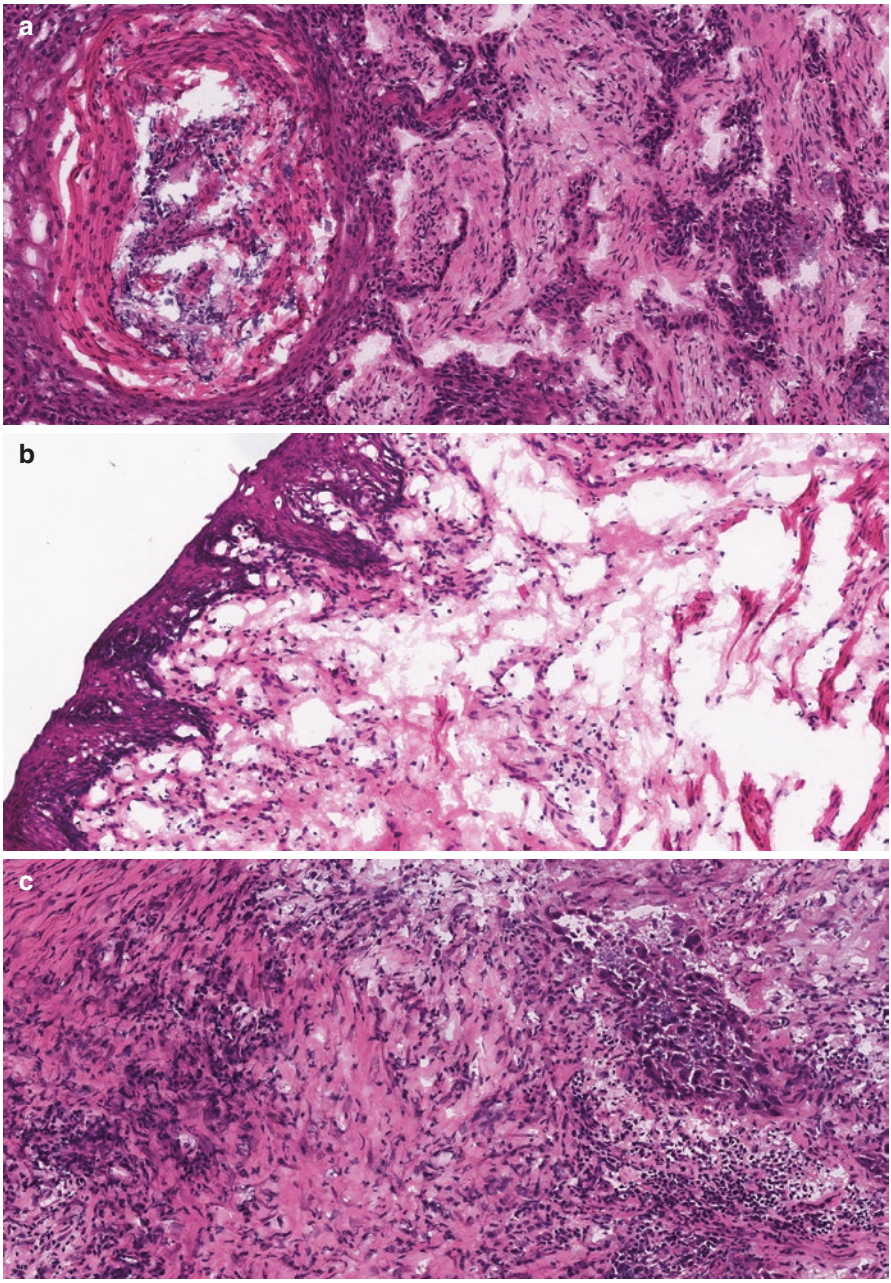

Fig. 2.4 Histological features of esophageal carcinoma prepared by sectioning of frozen tissues. The quality of the morphological features is inferior to those in Fig. 2.2 or 2.3. (**a**) Squamous cell carcinoma of esophagus. (**b**) Non-neoplastic esophageal epithelium (control in research). (**c**) Para-esophageal lymph node infiltrated by squamous cell carcinoma

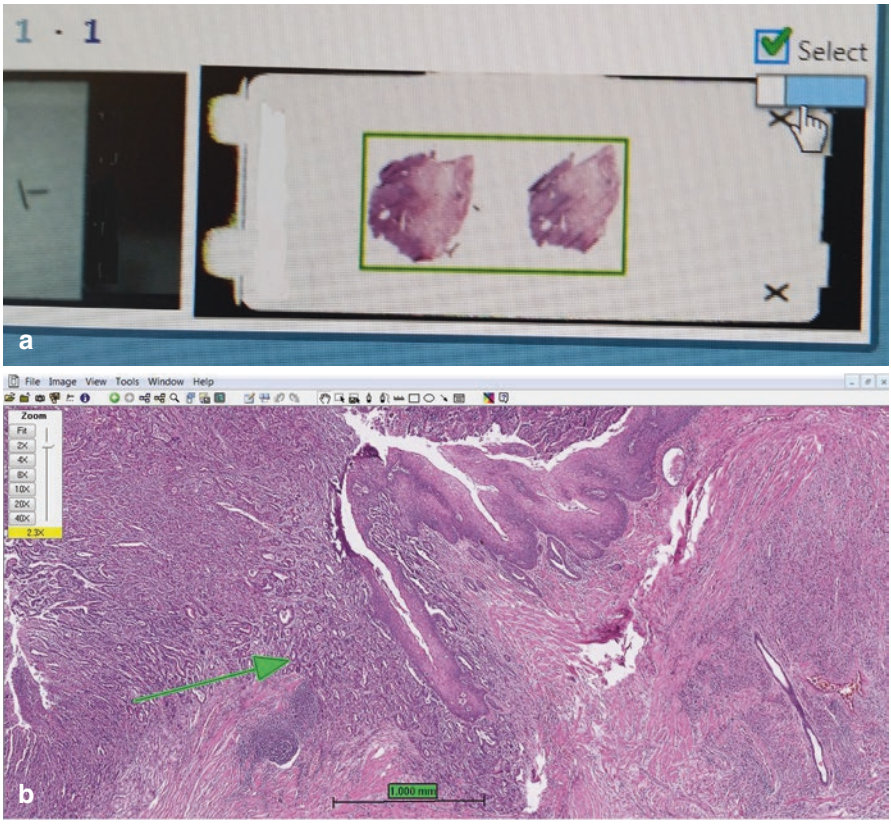

Fig. 2.5 Whole-slide imaging of esophageal carcinoma. (**a**) Capture of the histology of an esophageal squamous cell carcinoma frozen section by scanner. (**b**) Image obtained from scanning of an esophageal adenocarcinoma. Arrow and scale are indicated. Zooming of the image is possible as noted on the right upper corner

esophageal carcinoma, including inhibition of overexpressed oncogenes, blocking cell division by interfering with cyclins and related genes, and enhancing apoptosis by suppressing anti-apoptotic genes.

Cancer cell lines need the appropriate medium to grow. Cancer cell lines often grow without attaching to a surface and they can proliferate to a much higher density in a culture dish. The resulting transformed cancer cell lines, in reciprocal fashion, can often cause tumors if injected into a susceptible animal to generate an animal model. Cancer cells can be harvested from the animal and form a more stable cancer cell line. In esophageal cancers, some of the more commonly used cell lines are actually secondary cell lines. Cancer cell lines can allow functional studies to be performed. They can be stored in liquid nitrogen for an indefinite period and retain their viability when thawed.

In esophageal cancers, there are published cancer cell lines available for both adenocarcinoma and squamous carcinoma [143–146]. When compared to esophageal squamous cell carcinoma, esophageal adenocarcinoma is relatively uniform in characteristics as the risk factors and pathogenesis are more established. Model research on esophageal adenocarcinoma relies almost entirely on a relatively small set of established cancer cell lines. The high genomic similarities between the esophageal cell lines and their original cancers provide rationale for their use. Nonetheless, cancer cell lines nearly always differ in important ways from the original cancer from which they were derived.

Animal Models

Animal models are important to study the effects of cancer in vivo and for the production of cancer cell lines. An animal model may be a clinically relevant application for developing therapeutic strategies. Cancer development is a complex process involving the accumulation of genetic alterations and their downstream effects as well as interactions with the microenvironment in different tissues. The cancer microenvironment and its interactions with the cancer are important in determining the growth dynamics of different cancers.

Injection of cancer or cancerous cells in the subcutaneous tissue of the skin of immunodeficient mice is a common practice to produce a cancer model in animals (Fig. 2.6a). In many instances, researchers use a cancer cell line as it is easy to grow. However, to adopt a personalized approach for testing the cancer from a particular group of patients, injection of cancer tissue is required which is labelled as patient-derived xenograft (PDX) model. This approach requires careful planning and highly experience personnel, and there is a high failure rate of growth of the tumor in the animal (when compared to using commercially obtained cancer cell lines).

In esophageal cancers, this approach cannot recapitulate the microenvironment of the esophagus or the response to targeting carcinogens. One approach is to generate an orthotopic (occurring at a normal site) model for esophageal carcinoma [147, 148] (Fig. 2.6b). The orthotopic model provides the optimum environment for cancer growth and drug testing. In the anatomical setting of esophageal cancer, the site is very difficult to approach surgically. Several approaches have been explored, but most of these have some shortcomings. The establishment of these orthotopic models needs to involve radiological guidance (magnetic resonance imaging and fluorescence imaging) so the cancer and the metastases can be visualized in real time [149]. In addition, pathological examination is important to clarify the histological typing, microscopic location, and microenvironment of the cancer in the animal.

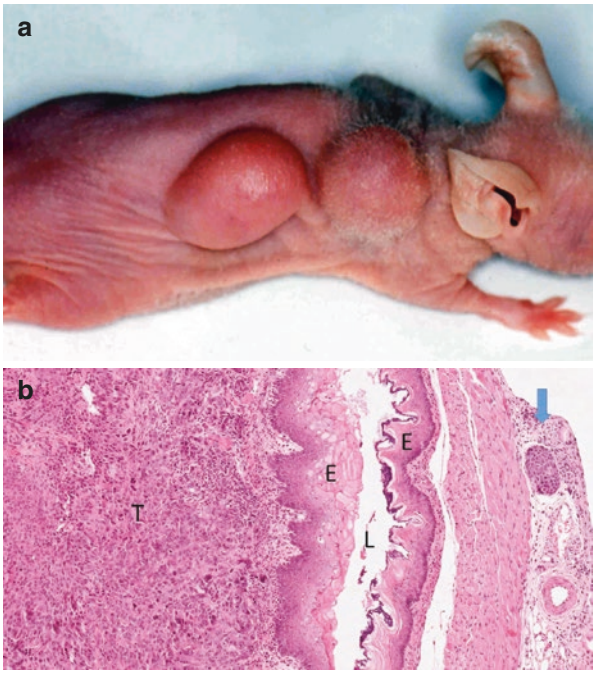

Fig. 2.6 Animal models of esophageal carcinoma. (**a**) Tumor produced in an immunodeficient mouse after subcutaneous injection of primary esophageal squamous cell carcinoma from a patient (courtesy of Dr. Johnny Tang from eHealth Sytle Biotechnology Limited, Hong Kong). (**b**) An orthotopic nude model of esophageal squamous cell carcinoma (courtesy of Professor Maria Lung from the University of Hong Kong, Hong Kong). Histological section of mouse esophagus showing the successful growth of a squamous cell carcinoma (from a cancer cell line surgically implanted in the wall of the esophagus) in the esophagus of the mouse. A carcinoma nodule is present in the lymphatic in the wall of the esophagus (arrow). L: lumen in the esophagus. E: esophageal epithelium. T: carcinoma

References

1. Lam KY, Ma L. Pathology of esophageal cancers: local experience and current insights. Chin Med J. 1997;110:459–64.
2. Lam AKY. Critical review: molecular biology of esophageal squamous cell carcinoma. Crit Rev Oncol Hematol. 2000;33:71–90.
3. Lam AK. Histopathological assessment for esophageal adenocarcinoma. Methods Mol Biol. 2018;1756:67–76.
4. Lam KY, Law S, Tung PH, Wong J. Esophageal basaloid squamous cell carcinoma: an unique clinicopathological entity with telomerase activity as a prognostic indicator. J Pathol. 2001;195:435–42.

5. Lam KY, Law SYK, Loke SL, Fok M, Ma LT. Double sarcomatoid carcinomas of the esophagus. Pathol Res Pract. 1996;192:604–9.
6. Lam KY, Dickens P, Loke SL, Fok M, Ma L, Wong J. Squamous cell carcinoma of the esophagus with mucin-secreting component (mucoepidermoid carcinoma and adenosquamous cell carcinoma): a clinicopathologic study and a review of literature. Eur J Surg Oncol. 1994;20: 25–31.
7. Law SYK, Fok M, Lam KY, Loke SL, Ma LT, Wong J. Small cell carcinoma of the esophagus. Cancer. 1994;73:2894–9.
8. Chow V, Law S, Lam KY, Luk JM, Wong J. Telomerase activity in small cell esophageal carcinoma. Dis Esophagus. 2001;14:139–42.
9. Lam KY, Law S, Tung PH, Wong J. Esophageal small cell carcinoma: clinicopathologic parameters, p53 overexpression, proliferative marker, and their impact on pathogenesis. Arch Pathol Lab Med. 2000;124:228–33.
10. Lam AK. Introduction: esophageal adenocarcinoma: updates of current status. Methods Mol Biol. 1756;2018:1–6.
11. Juckett G, Hartman-Adams H. Human papillomavirus: clinical manifestations and prevention. Am Fam Physician. 2010;82:1209–13.
12. Husain N, Neyaz A. Human papillomavirus associated head and neck squamous cell carcinoma: controversies and new concepts. J Oral Biol Craniofac Res. 2017;7:198–205.
13. Shaikh MH, Khan AI, Sadat A, Chowdhury AH, Jinnah SA, Gopalan V, Lam AK, Clarke DTW, McMillan NAJ, Johnson NW. Prevalence and types of high-risk human papillomaviruses in head and neck cancers from Bangladesh. BMC Cancer. 2017;17:792.
14. He D, Zhang DK, Lam KY, Ma L, Ngan HYS, Liu SS, Tsao SW. Prevalence of HPV infection in esophageal squamous cell carcinoma in Chinese patients and its relationship to the p53 gene mutation. Int J Cancer. 1997;72:959–64.
15. Lam KY, He D, Ma L, Zhang D, Ngan HYS, Wan TSK, Tsao SW. Presence of human papillomavirus in esophageal squamous cell carcinomas of Hong Kong Chinese and its relationship with p53 gene mutation. Hum Pathol. 1997;28:657–63.
16. Li X, Gao C, Yang Y, Zhou F, Li M, Jin Q, Gao L. Systematic review with meta-analysis: the association between human papillomavirus infection and oesophageal cancer. Aliment Pharmacol Ther. 2014;39:270–81.
17. Rajendra S, Yang T, Xuan W, Sharma P, Pavey D, Lee CS, Le S, Collins J, Wang B. Active human papillomavirus involvement in Barrett's dysplasia and oesophageal adenocarcinoma is characterized by wild-type p53 and aberrations of the retinoblastoma protein pathway. Int J Cancer. 2017;141:2037–49.
18. Antonsson A, Knight L, Whiteman DC. Human papillomavirus not detected in esophageal adenocarcinoma tumor specimens. Cancer Epidemiol. 2016;41:96–8.
19. Löfdahl HE, Du J, Näsman A, Andersson E, Rubio CA, Lu Y, Ramqvist T, Dalianis T, Lagergren J, Dahlstrand H. Prevalence of human papillomavirus (HPV) in oesophageal squamous cell carcinoma in relation to anatomical site of the tumour. PLoS One. 2012;7:e46538.
20. Wang WJ, Wu MJ, Ren JL, Xie P, Chang J, Hu GM, Wu HF. p16INK4a is not a reliable screening marker of HPV infection in esophageal squamous cell carcinoma:evidence from a meta-analysis. Int J Biol Markers. 2016;31:e431–9.
21. Bucchi D, Stracci F, Buonora N, Masanotti G. Human papillomavirus and gastrointestinal cancer: a review. World J Gastroenterol. 2016;22:7415–30.
22. Xi R, Pan S, Chen X, Hui B, Zhang L, Fu S, Li X, Zhang X, Gong T, Guo J, Zhang X, Che S. HPV16 E6-E7 induces cancer stem-like cells phenotypes in esophageal squamous cell carcinoma through the activation of PI3K/Akt signaling pathway in vitro and in vivo. Oncotarget. 2016;7:57050–65.
23. Zhang D, Zhang W, Liu W, Mao Y, Fu Z, Liu J, Huang W, Zhang Z, An D, Li B. Human papillomavirus infection increases the chemoradiation response of esophageal squamous cell carcinoma based on P53 mutation. Radiother Oncol. 2017;124:155–60.
24. Wang WL, Wang YC, Lee CT, Chang CY, Lo JL, Kuo YH, Hsu YC, Mo LR. The impact of human papillomavirus infection on the survival and treatment response of patients with esophageal cancers. J Dig Dis. 2015;16:256–63.

25. da Costa AM, Fregnani JHTG, Pastrez PRA, Mariano VS, Silva EM, Neto CS, Guimarães DP, Villa LL, Sichero L, Syrjanen KJ, Longatto-Filho A. HPV infection and p53 and p16 expression in esophageal cancer: are they prognostic factors? Infect Agent Cancer. 2017;12:54.
26. Pouyanfard S, Müller M. Human papillomavirus first and second generation vaccines-current status and future directions. Biol Chem. 2017;398:871–89.
27. Al-Haddad S, El-Zimaity H, Hafezi-Bakhtiari S, Rajendra S, Streutker CJ, Vajpeyi R, Wang B. Infection and esophageal cancer. Ann N Y Acad Sci. 2014;1325:187–96.
28. Lam KY, Srivastava G, Leung ML, Ma L. Absence of Epstein-Barr virus in esophageal squamous cell carcinoma: a study of 74 cases using in-situ hybridization. J Clin Pathol Mol Pathol. 1995;48:M188–90.
29. Xu W, Liu Z, Bao Q, Qian Z. Viruses, other pathogenic microorganisms and esophageal cancer. Gastrointest Tumors. 2015;2:2–13.
30. Wang ZK, Yang YS. Upper gastrointestinal microbiota and digestive diseases. World J Gastroenterol. 2013;19:1541–50.
31. Gibson MK, Dhaliwal AS, Clemons NJ, Phillips WA, Dvorak K, Tong D, Law S, Pirchi ED, Räsänen J, Krasna MJ, Parikh K, Krishnadath KK, Chen Y, Griffiths L, Colleypriest BJ, Farrant JM, Tosh D, Das KM, Bajpai M. Barrett's esophagus: cancer and molecular biology. Ann N Y Acad Sci. 2013;1300:296–314.
32. Li B, Xu WW, Lam AKY, Wang Y, Hu HF, Guan XY, Qin YR, Saremi N, Tsao SW, He QY, Cheung ALM. Significance of PI3K/AKT signaling pathway in metastasis of esophageal squamous cell carcinoma and its potential as a target for anti-metastasis therapy. Oncotarget. 2017;8:38755–66.
33. Yu VZ, Wong VC, Dai W, Ko JM, Lam AK, Chan KW, Samant RS, Lung HL, Shuen WH, Law S, Chan YP, Lee NP, Tong DK, Law TT, Lee VH, Lung ML. Nuclear localization of DNAJB6 is associated with survival of patients with esophageal cancer and reduces AKT signaling and proliferation of cancer cells. Gastroenterology. 2015;149:1825–36.
34. Chung Y, Lam AK, Luk JM, Law S, Chan KW, Lee PY, Wong J. Altered E-cadherin expression and p120 catenin localization in esophageal squamous cell carcinoma. Ann Surg Oncol. 2007;14:3260–7.
35. Xu WW, Li B, Lam AK, Tsao SW, Law SY, Chan KW, Yuan QJ, Cheung AL. Targeting VEGFR1- and VEGFR2-expressing non-tumor cells is essential for esophageal cancer therapy. Oncotarget. 2015;6:1790–805.
36. Chan D, Tsoi MY, Liu CD, Chan SH, Law SY, Chan KW, Chan YP, Gopalan V, Lam AK, Tang JC. Oncogene GAEC1 regulates CAPN10 expression which predicts survival in esophageal squamous cell carcinoma. World J Gastroenterol. 2013;19:2772–80.
37. Chai AW, Cheung AK, Dai W, Ko JM, Ip JC, Chan KW, Kwong DL, Ng WT, Lee AW, Ngan RK, Yau CC, Tung SY, Lee VH, Lam AK, Pillai S, Law S, Lung ML. Metastasis-suppressing NID2, an epigenetically-silenced gene, in the pathogenesis of nasopharyngeal carcinoma and esophageal squamous cell carcinoma. Oncotarget. 2016;7:78859–71.
38. Lam KY, Tsao SW, Zhang D, Law S, He D, Ma L, Wong J. Prevalence and predictive value of p53 mutation in patients with esophageal squamous cell carcinomas: a prospective clinicopathological study and survival analysis of 70 patients. Int J Cancer. 1997;74:212–9.
39. Appelman HD, Matejcic M, Parker MI, Riddell RH, Salemme M, Swanson PE, Villanacci V. Progression of esophageal dysplasia to cancer. Ann N Y Acad Sci. 2014;1325:96–107.
40. Lee KTW, Gopalan V, Lam AK. Somatic DNA copy number alterations detection for oesophageal adenocarcinoma using digital polymerase chain reaction. Methods Mol Biol. 2018;1756:195–212.
41. Islam F, Tang JC, Gopalan V, Lam AK. Epigenetics: DNA methylation analysis in esophageal adenocarcinoma. Methods Mol Biol. 2018;1756:247–56.
42. Haque MH, Islam MN, Islam F, Gopalan V, Nguyen NT, Lam AK, Shiddiky MJ. Electrochemical detection of FAM134B mutations in oesophageal cancer based on DNA-gold affinity interactions. Electroanalysis. 2017;29:1359–67.
43. Haque MH, Gopalan V, Islam MN, Masud MK, Bhattacharjee R, Hossain MSA, Nguyen NT, Lam AK, Shiddiky MJA. Quantification of gene-specific DNA methylation in oesophageal cancer via electrochemistry. Anal Chim Acta. 2017;976:84–93.

44. Pack SD, Karkera JD, Zhuang Z, Pak ED, Balan KV, Hwu P, Park WS, Pham T, Ault DO, Glaser M, Liotta L, Detera-Wadleigh SD, Wadleigh RG. Molecular cytogenetic fingerprinting of esophageal squamous cell carcinoma by comparative genomic hybridization reveals a consistent pattern of chromosomal alterations. Genes Chromosomes Cancer. 1999;25:160–8.
45. Walch AK, Zitzelsberger HF, Bruch J, Keller G, Angermeier D, Aubele MM, Mueller J, Stein H, Braselmann H, Siewert JR, Höfler H, Werner M. Chromosomal imbalances in Barrett's adenocarcinoma and the metaplasia-dysplasia-carcinoma sequence. Am J Pathol. 2000;156:555–66.
46. Kwong D, Lam A, Guan X, Law S, Tai A, Wong J, Sham J. Chromosomal aberrations in esophageal squamous cell carcinoma among Chinese: gain of 12p predicts poor prognosis after surgery. Hum Pathol. 2004;35:309–16.
47. Qin YR, Wang LD, Fan ZM, Kwong D, Guan XY. Comparative genomic hybridization analysis of genetic aberrations associated with development of esophageal squamous cell carcinoma in Henan, China. World J Gastroenterol. 2008;14:1828–35.
48. Tang JCO, Lam KY, Law S, Wong J, Srivastava G. Detection of genetic alterations in esophageal squamous cell carcinomas and adjacent normal epithelia by comparative DNA fingerprinting using inter-simple sequence repeat PCR. Clin Cancer Res. 2001;7:1539–45.
49. Hu YC, Lam KY, Law S, Wong J, Srivastava G. Identification of differentially expressed in esophageal squamous cell carcinoma (ESCC) by cDNA expression array: overexpression of Fra-1, Neogenin, Id-1 and CDC25B genes in ESCC. Clin Cancer Res. 2001;7:2213–21.
50. Fatima S, Chui CH, Tang WK, Hui KS, Au HW, Li WY, Wong MM, Cheung F, Tsao SW, Lam KY, Beh PS, Wong J, Law S, Srivastava G, Ho KP, Chan AS, Tang JC. Transforming capacity of two novel genes JS-1 and JS-2 located in chromosome 5p and their overexpression in human esophageal squamous cell carcinoma. Int J Mol Med. 2006;17:159–70.
51. Tang WK, Chui CH, Fatima S, Kok SH, Pak KC, Ou TM, Hui KS, Wong MM, Wong J, Law S, Tsao SW, Lam KY, Beh PS, Srivastava G, Chan AS, Ho KP, Tang JC. Oncogenic properties of a novel gene JK-1 located in chromosome 5p and its overexpression in human esophageal squamous cell carcinoma. Int J Mol Med. 2007;19:915–23.
52. Goh XY, Rees JR, Paterson AL, Chin SF, Marioni JC, Save V, O'Donovan M, Eijk PP, Alderson D, Ylstra B, Caldas C, Fitzgerald RC. Integrative analysis of array-comparative genomic hybridisation and matched gene expression profiling data reveals novel genes with prognostic significance in oesophageal adenocarcinoma. Gut. 2011;60:1317–26.
53. Law FB, Chen YW, Wong KY, Ying J, Tao Q, Langford C, Lee PY, Law S, Cheung RW, Chui CH, Tsao SW, Lam KY, Wong J, Srivastava G, Tang JC. Identification of a novel tumor transforming gene GAEC1 at 7q22 which encodes a nuclear protein and is frequently amplified and overexpressed in esophageal squamous cell carcinoma. Oncogene. 2007;26:5877–88.
54. Haque MH, Gopalan V, Chan KW, Shiddiky MJ, Smith RA, Lam AK. Identification of novel FAM134B (JK1) mutations in oesophageal squamous cell carcinoma. Sci Rep. 2016;6:29173.
55. Lee KT, Smith RA, Gopalan V, Lam AK. Targeted single gene mutation in oesophageal adenocarcinoma. Methods Mol Biol. 1756;2018:213–29.
56. Pillai S, Gopalan V, Lam AK. DNA genome sequencing in oesophageal adenocarcinoma. Methods Mol Biol. 2018;1756:231–46.
57. Pillai S, Gopalan V, Lam AK. Review of sequencing platforms and their applications in phaeochromocytoma and paragangliomas. Crit Rev Oncol Hematol. 2017;116:58–67.
58. Lin DC, Hao JJ, Nagata Y, Xu L, Shang L, Meng X, Sato Y, Okuno Y, Varela AM, Ding LW, Garg M, Liu LZ, Yang H, Yin D, Shi ZZ, Jiang YY, Gu WY, Gong T, Zhang Y, Xu X, Kalid O, Shacham S, Ogawa S, Wang MR, Koeffler HP. Genomic and molecular characterization of esophageal squamous cell carcinoma. Nat Genet. 2014;46:467–73.
59. Wang Q, Bai J, Abliz A, Liu Y, Gong K, Li J, Shi W, Pan Y, Liu F, Lai S, Yang H, Lu C, Zhang L, Chen W, Xu R, Cai H, Ke Y, Zeng C. An old story retold: loss of G1 control defines a distinct genomic subtype of esophageal squamous cell carcinoma. Genomics Proteomics Bioinformatics. 2015;13:258–70.
60. Stachler MD, Taylor-Weiner A, Peng S, McKenna A, Agoston AT, Odze RD, Davison JM, Nason KS, Loda M, Leshchiner I, Stewart C, Stojanov P, Seepo S, Lawrence MS, Ferrer-Torres

D, Lin J, Chang AC, Gabriel SB, Lander ES, Beer DG, Getz G, Carter SL, Bass AJ. Paired exome analysis of Barrett's esophagus and adenocarcinoma. Nat Genet. 2015;47:1047–55.
61. Rajendra S, Wang B, Merrett N, Sharma P, Humphris J, Lee HC, Wu J. Genomic analysis of HPV-positive versus HPV-negative oesophageal adenocarcinoma identifies a differential mutational landscape. J Med Genet. 2016;53:227–31.
62. Findlay JM, Castro-Giner F, Makino S, Rayner E, Kartsonaki C, Cross W, Kovac M, Ulahannan D, Palles C, Gillies RS, MacGregor TP, Church D, Maynard ND, Buffa F, Cazier JB, Graham TA, Wang LM, Sharma RA, Middleton M, Tomlinson I. Differential clonal evolution in oesophageal cancers in response to neo-adjuvant chemotherapy. Nat Commun. 2016;7:11111.
63. Liu W, Snell JM, Jeck WR, Hoadley KA, Wilkerson MD, Parker JS, Patel N, Mlombe YB, Mulima G, Liomba NG, Wolf LL, Shores CG, Gopal S, Sharpless NE. Subtyping sub-Saharan esophageal squamous cell carcinoma by comprehensive molecular analysis. JCI Insight. 2016;1:e88755.
64. Hao JJ, Lin DC, Dinh HQ, Mayakonda A, Jiang YY, Chang C, Jiang Y, Lu CC, Shi ZZ, Xu X, Zhang Y, Cai Y, Wang JW, Zhan QM, Wei WQ, Berman BP, Wang MR, Koeffler HP. Spatial intratumoral heterogeneity and temporal clonal evolution in esophageal squamous cell carcinoma. Nat Genet. 2016;48:1500–7.
65. Chen XX, Zhong Q, Liu Y, Yan SM, Chen ZH, Jin SZ, Xia TL, Li RY, Zhou AJ, Su Z, Huang YH, Huang QT, Huang LY, Zhang X, Zhao YN, Yun JP, Wu QL, Lin DX, Bai F, Zeng MS. Genomic comparison of esophageal squamous cell carcinoma and its precursor lesions by multi-region whole-exome sequencing. Nat Commun. 2017;8:524.
66. Forouzanfar N, Baranova A, Milanizadeh S, Heravi-Moussavi A, Jebelli A, Abbaszadegan MR. Novel candidate genes may be possible predisposing factors revealed by whole exome sequencing in familial esophageal squamous cell carcinoma. Tumour Biol. 2017;39:1010428317699115.
67. Dai W, Ko JMY, Choi SSA, Yu Z, Ning L, Zheng H, Gopalan V, Chan KT, Lee NP, Chan KW, Law SY, Lam AK, Lung ML. Whole-exome sequencing reveals critical genes underlying metastasis in oesophageal squamous cell carcinoma. J Pathol. 2017;242:500–10.
68. Zhang J, Baran J, Cros A, Guberman JM, Haider S, Hsu J, Liang Y, Rivkin E, Wang J, Whitty B, Wong-Erasmus M, Yao L, Kasprzyk A. International cancer genome consortium data portal—a one-stop shop for cancer genomics data. Database (Oxford). 2011;2011:bar026.
69. Dulak AM, Stojanov P, Peng S, Lawrence MS, Fox C, Stewart C, Bandla S, Imamura Y, Schumacher SE, Shefler E, McKenna A, Carter SL, Cibulskis K, Sivachenko A, Saksena G, Voet D, Ramos AH, Auclair D, Thompson K, Sougnez C, Onofrio RC, Guiducci C, Beroukhim R, Zhou Z, Lin L, Lin J, Reddy R, Chang A, Landrenau R, Pennathur A, Ogino S, Luketich JD, Golub TR, Gabriel SB, Lander ES, Beer DG, Godfrey TE, Getz G, Bass AJ. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. Nat Genet. 2013;45:478–86.
70. Song Y, Li L, Ou Y, Gao Z, Li E, Li X, Zhang W, Wang J, Xu L, Zhou Y, Ma X, Liu L, Zhao Z, Huang X, Fan J, Dong L, Chen G, Ma L, Yang J, Chen L, He M, Li M, Zhuang X, Huang K, Qiu K, Yin G, Guo G, Feng Q, Chen P, Wu Z, Wu J, Ma L, Zhao J, Luo L, Fu M, Xu B, Chen B, Li Y, Tong T, Wang M, Liu Z, Lin D, Zhang X, Yang H, Wang J, Zhan Q. Identification of genomic alterations in oesophageal squamous cell cancer. Nature. 2014;509:91–5.
71. Nones K, Waddell N, Wayte N, Patch AM, Bailey P, Newell F, Holmes O, Fink JL, MCJ Q, Tang YH, Lampe G, Quek K, Loffler KA, Manning S, Idrisoglu S, Miller D, Xu Q, Waddell N, Wilson PJ, TJC B, Christ AN, Harliwong I, Nourse C, Nourbakhsh E, Anderson M, Kazakoff S, Leonard C, Wood S, Simpson PT, Reid LE, Krause L, Hussey DJ, Watson DI, Lord RV, Nancarrow D, Phillips WA, Gotley D, Smithers BM, Whiteman DC, Hayward NK, Campbell PJ, Pearson JV, Grimmond SM, Barbour AP. Genomic catastrophes frequently arise in esophageal adenocarcinoma and drive tumorigenesis. Nat Commun. 2014;5:5224.
72. Weaver JMJ, Ross-Innes CS, Shannon N, Lynch AG, Forshew T, Barbera M, Murtaza M, Ong CJ, Lao-Sirieix P, Dunning MJ, Smith L, Smith ML, Anderson CL, Carvalho B, O'Donovan M, Underwood TJ, May AP, Grehan N, Hardwick R, Davies J, Oloumi A, Aparicio S, Caldas C, Eldridge MD, PAW E, Rosenfeld N, Tavaré S, Fitzgerald RC, OCCAMS consortium.

Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. Nat Genet. 2014;46:837–43.
73. Paterson AL, Weaver JM, Eldridge MD, Tavaré S, Fitzgerald RC, Edwards PA, OCCAMs Consortium. Mobile element insertions are frequent in oesophageal adenocarcinomas and can mislead paired-end sequencing analysis. BMC Genomics. 2015;16:473.
74. Ross-Innes CS, Becq J, Warren A, Cheetham RK, Northen H, O'Donovan M, Malhotra S, di Pietro M, Ivakhno S, He M, Weaver JMJ, Lynch AG, Kingsbury Z, Ross M, Humphray S, Bentley D, Fitzgerald RC. Whole-genome sequencing provides new insights into the clonal architecture of Barrett's esophagus and esophageal adenocarcinoma. Nat Genet. 2015;47:1038–46.
75. Zhang L, Zhou Y, Cheng C, Cui H, Cheng L, Kong P, Wang J, Li Y, Chen W, Song B, Wang F, Jia Z, Li L, Li Y, Yang B, Liu J, Shi R, Bi Y, Zhang Y, Wang J, Zhao Z, Hu X, Yang J, Li H, Gao Z, Chen G, Huang X, Yang X, Wan S, Chen C, Li B, Tan Y, Chen L, He M, Xie S, Li X, Zhuang X, Wang M, Xia Z, Luo L, Ma J, Dong B, Zhao J, Song Y, Ou Y, Li E, Xu L, Wang J, Xi Y, Li G, Xu E, Liang J, Yang X, Guo J, Chen X, Zhang Y, Li Q, Liu L, Li Y, Zhang X, Yang H, Lin D, Cheng X, Guo Y, Wang J, Zhan Q, Cui Y. Genomic analyses reveal mutational signatures and frequently altered genes in esophageal squamous cell carcinoma. Am J Hum Genet. 2015;96:597–611.
76. Qin HD, Liao XY, Chen YB, Huang SY, Xue WQ, Li FF, Ge XS, Liu DQ, Cai Q, Long J, Li XZ, Hu YZ, Zhang SD, Zhang LJ, Lehrman B, Scott AF, Lin D, Zeng YX, Shugart YY, Jia WH. Genomic characterization of esophageal squamous cell carcinoma reveals critical genes underlying tumorigenesis and poor prognosis. Am J Hum Genet. 2016;98:709–27.
77. Sawada G, Niida A, Uchi R, Hirata H, Shimamura T, Suzuki Y, Shiraishi Y, Chiba K, Imoto S, Takahashi Y, Iwaya T, Sudo T, Hayashi T, Takai H, Kawasaki Y, Matsukawa T, Eguchi H, Sugimachi K, Tanaka F, Suzuki H, Yamamoto K, Ishii H, Shimizu M, Yamazaki H, Yamazaki M, Tachimori Y, Kajiyama Y, Natsugoe S, Fujita H, Mafune K, Tanaka Y, Kelsell DP, Scott CA, Tsuji S, Yachida S, Shibata T, Sugano S, Doki Y, Akiyama T, Aburatani H, Ogawa S, Miyano S, Mori M, Mimori K. Genomic landscape of esophageal squamous cell carcinoma in a Japanese population. Gastroenterology. 2016;150:1171–82.
78. Secrier M, Li X, de Silva N, Eldridge MD, Contino G, Bornschein J, MacRae S, Grehan N, O'Donovan M, Miremadi A, Yang TP, Bower L, Chettouh H, Crawte J, Galeano-Dalmau N, Grabowska A, Saunders J, Underwood T, Waddell N, Barbour AP, Nutzinger B, Achilleos A, Edwards PA, Lynch AG, Tavaré S, Fitzgerald RC, Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium. Mutational signatures in esophageal adenocarcinoma define etiologically distinct subgroups with therapeutic relevance. Nat Genet. 2016;48:1131–41.
79. Cheng C, Zhou Y, Li H, Xiong T, Li S, Bi Y, Kong P, Wang F, Cui H, Li Y, Fang X, Yan T, Li Y, Wang J, Yang B, Zhang L, Jia Z, Song B, Hu X, Yang J, Qiu H, Zhang G, Liu J, Xu E, Shi R, Zhang Y, Liu H, He C, Zhao Z, Qian Y, Rong R, Han Z, Zhang Y, Luo W, Wang J, Peng S, Yang X, Li X, Li L, Fang H, Liu X, Ma L, Chen Y, Guo S, Chen X, Xi Y, Li G, Liang J, Yang X, Guo J, Jia J, Li Q, Cheng X, Zhan Q, Cui Y. Whole-genome sequencing reveals diverse models of structural variations in esophageal squamous cell carcinoma. Am J Hum Genet. 2016;98:256–74.
80. Cheng C, Cui H, Zhang L, Jia Z, Song B, Wang F, Li Y, Liu J, Kong P, Shi R, Bi Y, Yang B, Wang J, Zhao Z, Zhang Y, Hu X, Yang J, He C, Zhao Z, Wang J, Xi Y, Xu E, Li G, Guo S, Chen Y, Yang X, Chen X, Liang J, Guo J, Cheng X, Wang C, Zhan Q, Cui Y. Genomic analyses reveal FAM84B and the NOTCH pathway are associated with the progression of esophageal squamous cell carcinoma. Gigascience. 2016;5:1.
81. Fels Elliott DR, Perner J, Li X, Symmons MF, Verstak B, Eldridge M, Bower L, O'Donovan M, Gay NJ, OCCAMS Consortium, Fitzgerald RC. Impact of mutations in toll-like receptor pathway genes on esophageal carcinogenesis. PLoS Genet. 2017;13:e1006808.
82. Noorani A, Bornschein J, Lynch AG, Secrier M, Achilleos A, Eldridge M, Bower L, Weaver JMJ, Crawte J, Ong CA, Shannon N, MacRae S, Grehan N, Nutzinger B, O'Donovan M, Hardwick R, Tavaré S. Fitzgerald RC; oesophageal cancer clinical and molecular stratification (OCCAMS) consortium. A comparative analysis of whole genome sequencing of esophageal adenocarcinoma pre- and post-chemotherapy. Genome Res. 2017;27:902–12.

83. Liu X, Zhang M, Ying S, Zhang C, Lin R, Zheng J, Zhang G, Tian D, Guo Y, Du C, Chen Y, Chen S, Su X, Ji J, Deng W, Li X, Qiu S, Yan R, Xu Z, Wang Y, Guo Y, Cui J, Zhuang S, Yu H, Zheng Q, Marom M, Sheng S, Zhang G, Hu S, Li R, Su M. Genetic alterations in esophageal tissues from squamous dysplasia to carcinoma. Gastroenterology. 2017;153: 166–77.
84. Mamoori A, Wahab R, Islam F, Lee K, Vider J, Lu CT, Gopalan V, Lam AK. Clinical and biological significance of miR-193a-3p targeted KRAS in colorectal cancer pathogenesis. Hum Pathol. 2018;71:145–56.
85. Mamoori A, Gopalan V, Lu CT, Chua TC, Morris DL, Smith RA, Lam AK. Expression pattern of miR-451 and its target MIF (macrophage migration inhibitory factor) in colorectal cancer. J Clin Pathol. 2017;70:308–12.
86. Pillai S, Lo CY, Liew V, Lalloz M, Smith RA, Gopalan V, Lam AK. microRNA 183 family profiles in pheochromocytomas are related to clinical parameters and SDHB expression. Hum Pathol. 2017;64:91–7.
87. Islam F, Gopalan V, Vider J, Wahab R, Ebrahimi F, Lu CT, Kasem K, Lam AKY. MicroRNA-186-5p overexpression modulates colon cancer growth by repressing the expression of the FAM134B tumour inhibitor. Exp Cell Res. 2017;357:260–70.
88. Lee KT, Tan JK, Lam AK, Gan SY. MicroRNAs serving as potential biomarkers and therapeutic targets in nasopharyngeal carcinoma: a critical review. Crit Rev Oncol Hematol. 2016;103:1–9.
89. Gopalan V, Smith RA, Lam AK. Downregulation of microRNA-498 in colorectal cancers and its cellular effects. Exp Cell Res. 2015;330:423–8.
90. Salajegheh A, Vosgha H, Md Rahman A, Amin M, Smith RA, Lam A. Modulatory role of miR-205 in angiogenesis and progression of thyroid cancer. J Mol Endocrinol. 2015;55: 183–96.
91. Ebrahimi F, Gopalan V, Wahab R, Lu CT, Anthony Smith R, Lam AK. Deregulation of miR-126 expression in colorectal cancer pathogenesis and its clinical significance. Exp Cell Res. 2015;339:333–41.
92. Gopalan V, Pillai S, Ebrahimi F, Salajegheh A, Lam TC, Le TK, Langsford N, Ho YH, Smith RA, Lam AK. Regulation of microRNA-1288 in colorectal cancer: altered expression and its clinicopathological significance. Mol Carcinog. 2014;53:E36–44.
93. Ebrahimi F, Gopalan V, Smith RA, Lam AK. miR-126 in human cancers: clinical roles and current perspectives. Exp Mol Pathol. 2014;96:98–107.
94. Maroof H, Salajegheh A, Smith RA, Lam AK. Role of microRNA-34 family in cancer with particular reference to cancer angiogenesis. Exp Mol Pathol. 2014;97:298–304.
95. Vosgha H, Salajegheh A, Smith RA, Lam AK. The important roles of miR-205 in normal physiology, cancers and as a potential therapeutic target. Curr Cancer Drug Targets. 2014;14:621–37.
96. Maroof H, Salajegheh A, Smith RA, Lam AK. MicroRNA-34 family, mechanisms of action in cancer: a review. Curr Cancer Drug Targets. 2014;14:737–51.
97. Amin M, Islam F, Gopalan V, Lam AK. Detection and quantification of microRNAs in in oesophageal adenocarcinoma. Methods Mol Biol. 1756;2018:257–68.
98. Kamal Masud M, Islam MN, Haque MH, Tanaka S, Gopalan V, Alici G, Nguyen NT, Lam AK, Hossain MSA, Yamauchi Y, Shiddiky MJA. Gold-loaded nanoporous superparamagnetic nanocubes for catalytic signal amplification in detecting miRNA. Chem Commun (Camb). 2017;53:8231–4.
99. Amin M, Lam AK. Current perspectives of mi-RNA in oesophageal adenocarcinoma: roles in predicting carcinogenesis, progression and values in clinical management. Exp Mol Pathol. 2015;98:411–8.
100. Mei LL, Qiu YT, Zhang B, Shi ZZ. MicroRNAs in esophageal squamous cell carcinoma: potential biomarkers and therapeutic targets. Cancer Biomark. 2017;19:1–9.
101. Islam F, Gopalan V, Law S, Tang JC, Chan KW. Lam AK∗. MiR-498 in oesophageal squamous cell carcinoma: clinicopathological impacts and functional interactions. Hum Pathol. 2017;62:141–51.

102. Gopalan V, Islam F, Pillai S, Tang JC, Tong DK, Law S, Chan KW, Lam AK. Overexpression of microRNA-1288 in oesophageal squamous cell carcinoma. Exp Cell Res. 2016;348:146–54.
103. Islam F, Qiao B, Smith RA, Gopalan V, Lam AK. Cancer stem cell: fundamental experimental pathological concepts and updates. Exp Mol Pathol. 2015;98:184–91.
104. Wahab SMR, Islam F, Gopalan V, Lam AK. The identifications and clinical implications of cancer stem cells in colorectal cancer. Clin Colorectal Cancer. 2017;16:93–102.
105. Islam F, Gopalan V, Smith RA, Lam AK. Translational potential of cancer stem cells: a review of the detection of cancer stem cells and their roles in cancer recurrence and cancer treatment. Exp Cell Res. 2015;335:135–47.
106. Chruścik A, Gopalan V, Lam AK. The clinical and biological roles of transforming growth factor beta in colon cancer stem cells: a systematic review. Eur J Cell Biol. 2018;97:15–22.
107. Qiao B, Gopalan V, Chen Z, Smith RA, Tao Q, Lam AKY. Epithelial-mesenchymal transition and mesenchymal-epithelial transition are essential for the acquisition of stem cell properties in hTERT-immortalised oral epithelial cells. Bio Cell. 2012;104:476–89.
108. Gopalan V, Islam F, Lam AK. Surface markers for the identification of cancer stem cells. Methods Mol Biol. 1692;2018:17–29.
109. Islam F, Gopalan V, Wahab R, Smith RA, Lam AK. Cancer stem cells in oesophageal squamous cell carcinoma: identification, prognostic and treatment perspectives. Crit Rev Oncol Hematol. 2015;96:9–19.
110. Islam F, Gopalan V, Lam AK. Identification of cancer stem cells in esophageal adenocarcinoma. Methods Mol Biol. 2018;1756:165–76.
111. di Pietro M, Alzoubaidi D, Fitzgerald RC. Barrett's esophagus and cancer risk: how research advances can impact clinical practice. Gut Liver. 2014;8:356–70.
112. Ong CAJ, Lao-Sirieix P, Fitzgerald RC. Biomarkers in Barrett's esophagus and esophageal adenocarcinoma: predictors of progression and prognosis. World J Gastroenterol. 2010;16:5669–81.
113. Chen M, Huang J, Zhu Z, Zhang J, Li K. Systematic review and meta-analysis of tumor biomarkers in predicting prognosis in esophageal cancer. BMC Cancer. 2013;13:539.
114. Shang L, Liu HJ, Hao JJ, Jiang YY, Shi F, Zhang Y, Cai Y, Xu X, Jia XM, Zhan QM, Wang MR. A panel of overexpressed proteins for prognosis in esophageal squamous cell carcinoma. PLoS One. 2014;9:e111045.
115. Lam KY, Law S, Lo T, Tung HM, Wong J. The clinicopathological significance of p21 and p53 expression in esophageal squamous cell carcinoma: an analysis of 153 patients. Am J Gastroenterol. 1999;94:2060–8.
116. Lam KY, Law SYK, So MKP, Fok M, Ma LT, Wong J. Prognostic implication of proliferative markers MIB-1 and PC 10 in esophageal squamous cell carcinoma. Cancer. 1996;77:7–13.
117. Tong DK, Law S, Kwong DL, Chan KW, Lam AK, Wong KH. Histological regression of squamous esophageal carcinoma assessed by percentage of residual viable cells after neoadjuvant chemoradiation is an important prognostic factor. Ann Surg Oncol. 2010;17:2184–92.
118. Lam KY, Law S, Ma LT, Ong SK, Wong J. Pre-operative chemotherapy for squamous cell carcinoma of the esophagus: do histological assessment and p53 overexpression predict chemo-responsiveness? Eur J Cancer. 1997;33:1221–5.
119. Okumura H, Uchikado Y, Setoyama T, Matsumoto M, Owaki T, Ishigami S, Natsugoe S. Biomarkers for predicting the response of esophageal squamous cell carcinoma to neoadjuvant chemoradiation therapy. Surg Today. 2014;44:421–8.
120. Zhang SS, Huang QY, Yang H, Xie X, Luo KJ, Wen J, Cai XL, Yang F, Hu Y, Fu JH. Correlation of p53 status with the response to chemotherapy-based treatment in esophageal cancer: a meta-analysis. Ann Surg Oncol. 2013;20:2419–27.
121. Bain GH, Petty RD. Predicting response to treatment in gastroesophageal junction adenocarcinomas: combining clinical, imaging, and molecular biomarkers. Oncologist. 2010;15:270–84.
122. Imdahl A, Jenkner J, Ihling C, Rückauer K, Farthmann EH. Is MIB-1 proliferation index a predictor for response to neoadjuvant therapy in patients with esophageal cancer? Am J Surg. 2000;179:514–20.

123. Lam AK. Application of pathological staging in esophageal adenocarcinoma. Methods Mol Biol. 2018;1756:93–103.
124. Ung L, Lam AK, Morris DL, Chua TC. Tissue-based biomarkers predicting outcomes in metastatic colorectal cancer: a review. Clin Transl Oncol. 2014;16:425–35.
125. Pakneshan S, Salajegheh A, Smith RA, Lam AK. Clinicopathological relevance of BRAF mutations in human cancer. Pathology. 2013;45:346–56.
126. Rahman MA, Salajegheh A, Smith RA, Lam AK. BRAF inhibitors: from the laboratory to clinical trials. Crit Rev Oncol Hematol. 2014;90:220–32.
127. Rahman MA, Salajegheh A, Smith RA, Lam AK. BRAF inhibitor therapy for melanoma, thyroid and colorectal cancers: development of resistance and future prospects. Curr Cancer Drug Targets. 2014;14:128–43.
128. O'Sullivan CC, Connolly RM. Pertuzumab and its accelerated approval: evolving treatment paradigms and new challenges in the management of HER2-positive breast cancer. Oncology (Williston Park). 2014;28:186–94.
129. Wiedmann MW, Mössner J. New and emerging combination therapies for esophageal cancer. Cancer Manag Res. 2013;5:133–46.
130. Kordes S, Cats A, Meijer SL, van Laarhoven HW. Targeted therapy for advanced esophagogastric adenocarcinoma. Crit Rev Oncol Hematol. 2014;90:68–76.
131. Orditura M, Galizia G, Fabozzi A, Lieto E, Gambardella V, Morgillo F, Del Genio GM, Fei L, Di Martino N, Renda A, Ciardiello F, De Vita F. Preoperative treatment of locally advanced esophageal carcinoma (review). Int J Oncol. 2013;43:1745–53.
132. Nakajima M, Kato H. Treatment options for esophageal squamous cell carcinoma. Expert Opin Pharmacother. 2013;14:1345–54.
133. Boland PM, Burtness B. Esophageal carcinoma: are modern targeted therapies shaking the rock? Curr Opin Oncol. 2013;25:417–24.
134. Lam KY, Tin L, Ma L. C-erbB-2 protein expression in oesophageal squamous epithelium from oesophageal squamous cell carcinomas, with special reference to histological grade of carcinoma and pre-invasive lesions. Eur J Surg Oncol. 1998;24:431–5.
135. Hicks DG, Whitney-Miller C. HER2 testing in gastric and gastroesophageal junction cancers: a new therapeutic target and diagnostic challenge. Appl Immunohistochem Mol Morphol. 2011;19:506–8.
136. Bartley AN, Washington MK, Colasacco C, Ventura CB, Ismaila N, Benson AB 3rd, Carrato A, Gulley ML, Jain D, Kakar S, Mackay HJ, Streutker C, Tang L, Troxell M, Ajani JA. HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology. J Clin Oncol. 2017;35:446–64.
137. Kumarasinghe MP, Brown I, Raftopoulos S, Bourke MJ, Charlton A, de Boer WB, Eckstein R, Epari K, Gill AJ, Lam AK, Price T, Streutker C, Lauwers GY. Standardised reporting protocol for endoscopic resection for Barrett oesophagus associated neoplasia: expert consensus recommendations. Pathology. 2014;46:473–80.
138. Saremi N, Lam AK. Application of tissue microarray in esophageal adenocarcinoma. In: Lam AK, editor. Methods in molecular biology: esophageal adenocarcinoma: Springer; 2018. (in press).
139. Lam AK, Leung M. Whole-slide imaging for esophageal adenocarcinoma. Methods Mol Biol. 2018;1756:135–42.
140. Gopalan V, Lam AK. Circulatory tumor cells in esophageal adenocarcinoma. Methods Mol Biol. 2018;1756:177–86.
141. Smith RA, Lam AK. Liquid biopsy for investigation of cancer DNA in oesophageal adenocarcinoma: cell free plasma DNA and exosome associated DNA. Methods Mol Biol. 2018;1756:187–94.
142. Islam F, Gopalan V. Lam AK. RNA interference mediated genes silencing in oesophageal adenocarcinoma. Methods Mol Biol. 2018;1756:269–79.
143. Tang JCO, Wan TSK, Wong N, Pang E, Lam KY, Law SYK, Chow LMC, Ma ESK, Chan LC, Wong J, Srivastava G. Establishment and characterization of a new xenograft-derived

human esophageal squamous cell line SLMT-1 of Chinese origin. Cancer Genet Cytogenet. 2001;124:36–41.
144. Hu YC, Lam KY, Wan TSK, Fang WG, Ma ESK, Chan LC, Srivastava G. Establishment and characterization of HKESC-1, a new cancer cell line from human esophageal squamous cell carcinoma. Cancer Genet Cytogenet. 2000;118:112–20.
145. Hu YC, Lam KY, Law SYK, Wan TSK, Ma ESK, Kwong YL, Chan LC, Wong J, Srivastava G. Establishment, characterization, karyotyping, and comparative genomic hybridization analysis of HKESC-2 and HKESC-3: two newly established human esophageal squamous cell lines. Cancer Genet Cytogenet. 2002;135:120–7.
146. Boonstra JJ, Tilanus HW, Dinjens WN. Translational research on esophageal adenocarcinoma: from cell line to clinic. Dis Esophagus. 2015;28:90–6.
147. Ip JC, Ko JM, Yu VZ, Chan KW, Lam AK, Law S, Tong DK, Lung ML. A versatile orthotopic nude mouse model for study of esophageal squamous cell carcinoma. Biomed Res Int. 2015;2015:910715.
148. Furihata T, Sakai T, Kawamata H, Omotehara F, Shinagawa Y, Imura J, Ueda Y, Kubota K, Fujimori T. A new in vivo model for studying invasion and metastasis of esophageal squamous cell carcinoma. Int J Oncol. 2001;19:903–7.
149. Hori T, Yamashita Y, Ohira M, Matsumura Y, Muguruma K, Hirakawa K. A novel orthotopic implantation model of human esophageal carcinoma in nude rats: CD44H mediates cancer cell invasion in vitro and in vivo. Int J Cancer. 2001;92:489–96.