Chapter 1

Introductory Chapter: Esophagus and Esophageal Cancer

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1. Introduction

Our body obtains nutritional supplies from the environment through two primary pipes: bronchus and esophagus, the two simplest organs in the respiratory and digestive systems, respectively. While the bronchus passes oxygen to the lung and expels carbon dioxide out of our biological system, the esophagus transports water and food into the stomach from where a sophisticated process of digestion and nutrient extraction begins.

Although skin cancer might be the most common malignancy in the world, accounting for at least 40% of all cancer cases [1], it is usually excluded from the annual cancer report due to its least perniciousness. The remainders are mostly found in the respiratory and digestive systems, particularly in the digestive system, which hides about twice as much cancer as found in the respiratory system. Five of the top 10 deadliest cancers take place in the digestive organs, including stomach, liver, colon, pancreas, and esophagus. Esophageal cancer is ranked as No. 6 on the list. While the incidence of most cancers is declining year by year, esophageal cancer continues climbing as the fast growing malignancy in the world. Based on a recent prediction, by the year of 2035, the global population of the esophageal cancer patients will be up by 77.4% [2]. In some regions of Asia, Africa, and the South America, the numbers could be doubled in less than 20 years. Logically, esophageal malignancy will very likely become one of the top global concerns in the near future.

The human esophagus is just a short tube of ~25 cm, separating from the rest of the body by two muscular rings at the ends, the upper esophageal sphincter and the lower esophageal sphincter, which control the flow of ingested materials from the mouth to the stomach. Like a corridor that sends visitors from the gate to the main building, the esophagus sends food from the mouth to the stomach. It is a rather simple structure, but, because of its critical location, any abnormalities associated with this organ can be devastating.

When food is being swallowed, the upper sphincter relaxes, allowing food to enter the pipe. Peristaltic contractions of the esophageal muscle push the food down through the lower sphincter into the stomach. Besides controlling the amount of swallowed food going down into the stomach, the lower esophageal sphincter also works like a dam sitting in between the esophagus and the stomach to prevent the stomach contents to back up into the esophagus. When this muscular structure does not hold well, gastroesophageal reflux disease (GERD) occurs, in which case stomach acid mixed with duodenal content flows back into the esophagus. If this happens frequently enough, it leads to esophagitis, and then to Barret's esophagus, a premalignant metaplasia of the esophageal lining changing from stratified squamous epithelium to simple columnar epithelium. Compared to normal people, individuals with Barret's esophagus can have as high as a 400-fold increased risk to develop esophageal cancer [3].

2. Epidemiology of esophageal cancer

Esophageal cancer is the ninth most common malignancy in the world. Most of the cases are either squamous cell carcinoma (ESCC) or adenocarcinoma (EAC). The former is the predominant one, accounting for ~90% of the cases. ESCC occurs in the squamous cell lining of the middle section of the esophagus and is more often found in Asia and Africa. China alone is responsible for more than 50% of the patient population. EAC, on the other hand, takes place in the cuboidal cells of the esophageal glands near the gastroesophageal junction and has been growing rapidly in western countries in recent years. Both types of esophageal cancer happen more often in males than in females, and the overall ratios of male to female are approximately 2.5 for ESCC and 4.4 for EAC. At the first look, the incidence of esophageal cancer seems to be geographic-related, as ESCC is more seen in Asia and Africa while EAC more common in Europe and North America. However, if we analyze the data further, we notice that the issue is actually more ethnic rather than geographic. Take a look at the cases in the United States, where ESCC incidence is found 4.8 times higher in Asian- and African Americans than in Caucasians, while EAC is just the opposite, 5 times higher in Caucasians than in other Americans [2]. Apparently, these two diseases selectively adhere to certain races of people regardless of where they live. This notion is also supported by the data from China, where ESCC patient population is 77 times greater than that of EAC [4]. Apparently, after a long history of sharing residential resources, each ethnic group has formed its unique life habits. For this reason, they tend to develop common health problems.

As far as we know today, smoking is the No. 1 risk factor for ESCC, particularly when it is in conjunction with drinking. A study found that ESCC incidence increased 12-fold in males and 19-fold in females in the population who use tobacco and alcohol together, compared to those who have one of the hobbies alone [5]. This connection can be easily seen in China, where the tobacco consumption is the highest in the world, higher than all other developing countries combined [6]. The Chinese also consume a lot of alcohol, particularly in northern and central provinces, where the ESCC incidence can reach 0.8% of the local residential population [1]. Here is the east end of the so-called "esophageal cancer belt." This association is also reflected by the data on the American males of Asian and African origins, who tend to smoke and drink abreast, thus making up for 90% of the ESCC patient population in the United States [7].

While the use of tobacco and alcohol together has been the main risk factor for ESCC, obesity and low vegetable consumption increase the chances to develop EAC. In the obese community, the excessive body weight puts constant pressure on the stomach and causes frequent acid reflux. These highly acidic fluids regurgitated from the stomach or even from the duodenum induce inflammation in the esophagus. As the episodes continue, the epithelial lining of the esophagus gradually transforms from stratified squamous epithelium to intestinal columnar phenotype for adaptive protection, as the latter is more endurable to acidic insults. Unfortunately, however, this metaplasia confers a greater danger to become malignant. Studies have shown that people with this kind of esophageal adaptation could have 400 times more likelihood to develop EAC than the general population [8]. Insufficient uptake of fresh fruits and vegetables can also create this type of drama.

Although both ESCC and EAC take place in this short organ, they are very different cancers. From an epidemiological point of view, there is only one common feature between ESCC and EAC, and that is both preferring men over women, while differences are a lot greater.

3. Genetics of esophageal cancer

In addition to the factors associated to life habits, there are also genetic elements contributing to esophageal cancer development. The genomic analysis reveals distinct profiles between ESCC and EAC. ESCC is more similar to squamous cell carcinoma of the head and neck than to EAC, while the latter has more resemblance to gastric adenocarcinoma.

ESCC is believed to develop from basal cell hyperplasia and dysplasia. During this process, the main mutation pattern is C to A substitution, which is commonly found in smokers [9]. The most frequently mutated genes include TP53 (p53, tumor suppression transcription factor), CDKN2A (p16, cyclin-dependent kinase inhibitor), KDM6A (histone demethylase), KMT2D (lysine methyltransferase), and RB1 (retinoblastoma-associated protein). On the other hand, some genes are highly expressed, such as CCND1 (cyclin D1), TP63 (tumor protein), SOX2 (sex-determining region Y), MYC (c-myc), FGFR1 (fibroblast growth factor receptor), TNFAIP3 (tumor necrosis factor-induced protein), and CHN (chimerin) [10]. **Table 1** lists the top five genes frequently mutated and the top five highly expressed.

EAC, on the other hand, is generally believed to originate from Barret's esophagus, an esophageal metaplasia in response to chronic acid reflux. During this process, esophageal epithelium transforms from a multilayer of squamous epithelial cells to a single layer of intestinal columnar epithelial cells, or like the metaphor used by Ahrens et al.: "turning skyscrapers into townhouses" [14]. The natural question is where the columnar cells come from. There are four theories currently to explain the origin of esophageal columnar cells: (1) true transdifferentiation of the esophageal squamous cells, (2) trans-commitment of the esophageal stem cells, (3) colonization and subsequent trans-commitment of bone marrow stem cells, and (4) replacement by gastric columnar epithelial cells.

The first theory is supported by the fact that the esophagus derives from the columnar epithelial cells initially during embryonic development and later is replaced by squamous

Gene	Protein	Function	Up (+)/down (−) in ESCC	References
TP53	p53	Tumor suppression, cellular stress response, cell death	—	[11]
CDKN2A	p16	Stabilizing p53	—	[11, 12]
KDM6A	Lysine demethylase 6A	Chromatin remodeling	—	[9]
KMT2D	Lysine methyltransferase 2D	Chromatin remodeling	—	[9]
RB1	Retinoblastoma transcriptional corepressor 1	Tumor suppression	—	[10]
CCND1	Cyclin D1	Cell cycle progression	+	[9]
TP63	p63	Transcription regulator	+	[9]
SOX2	Sex determining region Y 2	Embryogenesis, stem cell maintenance	+	[9]
MYC	c-myc	Cell cycle progression, cell transformation, apoptosis	+	[9]
EGFR	EGF receptor	Mediating EGF signaling	+	[13]

Table 1. The top five of the most commonly mutated genes (−) and the top five of the most highly expressed genes (+) in ESCC.

epithelium [15]. Therefore, the differentiated esophageal squamous epithelial cells might be able to transform back to columnar cells. Does it sound possible? However, *in vitro* study demonstrated that acid and/or bile treatment upregulated CDX-2 expression in normal esophageal epithelial cells (Het1A), which led to intestinal phenotype [16]. The esophageal epithelium is maintained by a distinct group of p63-expressing stem cells beneath the mucosa under the influence of a specific cue within the organ. When the esophagus is insulted by acidic refluxate repeatedly, the progenitor cells lose p63 expression and are misled to differentiate into columnar instead of squamous epithelial cells. This is where the second theory stands [17]. Does it sound reasonable? More evidence is needed. The third theory seems pretty strong, because it is supported by experimental evidence. In 2008, Sarosi et al. successfully transplanted female rats that had been surgically induced by reflux esophagitis with bone marrow from male rats and later identified Y-chromosome in half of the esophageal epithelial cell population, indicating a colonization of bone marrow stem cells in esophageal epithelium [18]. However, Aikou et al. conducted similar experiment using mice and could not confirm bone marrow-derived metaplastic esophageal epithelial cells [19]. Nevertheless, both the second and third theories recognize the important contribution from stem cells during metaplastic transformation. The only difference is the origin of progenitor cells. If bone marrow stem cells could be misguided to differentiate into columnar phenotype where squamous cells are supposed to be, why could not the local stem cells? The fourth theory has also found experimental evidence in human. In 2014, Lavery et al. showed that labeled gastric cells residing in the middle of esophageal glands had undergone metaplastic transformation, suggesting that esophageal columnar epithelial cells could be from the migration of gastric cardiac columnar epithelial cells [20].

The next question is how esophageal metaplasia turns into EAC. There are two main theories currently. The first theory thinks that EAC develops from Barret's esophagus through a stepwise

Gene	Protein	Function	Up (+)/down (−) in EAC	References
TP53	p53	Tumor suppression, cellular stress response, cell death	—	[21]
CDKN2A	p16	Stabilizing p53	—	[22]
SMAD4	Smad 4	Tumor suppression	—	[23]
SYNE1	Spectrin repeat containing nuclear envelope protein 1	Organelle movement	—	[24]
DOCK2	Dedicator of cytokinesis 2	Cell migration	—	[24]
MYC	c-myc	Cell cycle progression, cell transformation, apoptosis	+	[25]
ERBB2	Her2	Stabilizing EGF binding to its receptor	+	[9]
GATA6	GATA binding protein 6	Cell differentiation in gut	+	[26]
VEGFA	Vascular endothelial growth factor A	Angiogenesis	+	[25]
CCNE1	Cyclin E1	Cell cycle progression	+	[9]

Table 2. The top five of the most commonly mutated genes (−) and the top five of the most highly expressed genes (+) in EAC.

accumulation of gene mutations. EAC is one of cancers with a high rate of gene mutation. In 2013, a study conducted by Dulak et al. performed whole-genome sequence analysis on 149 pairs of EAC versus normal tissue samples and identified a total of 17,383 mutations in 8331 genes in EAC specimens, including 16,516 non-silent mutations and 1954 insertion–deletion-null mutations [24]. Of these genes, 26 were significantly mutated. As seen in ESCC, TP53 and CDKN2A were on top of the list. One of the differences, however, is A to C base transversion that is more common in EAC while C to A is more common in ESCC. The second theory involves a massive chromosomal instability due to the inactivation of p53 and p16. Loss of TP53 has been shown to increase the possibility of malignance by 16-fold [21]. Without functional p53, aneuploidy develops, which increases the pace of genome doubling. CDKN2A is the gene coding for p16, a cyclin-dependent kinase inhibitor that can sequester MDM2 and thereby prevent p53 being degraded. Inactivation of CDKN2A can be interpreted as a reinforcement to the elimination of p53. **Table 2** lists the top five mutated genes and top five overexpressed genes associated with EAC.

4. The guardian of the genome: p53

Based on multiple genetic analyses performed by several independent groups [9, 27–29], TP53 always appeared to be the most frequently mutated gene in both ESCC and EAC. Not just esophageal malignancy, 50–60% of human cancers that have been studied so far contain homozygous mutations in TP53 [30]. That is almost to say, p53 has to be disabled in order to turn a normal cell into a cancerous cell. Why is p53 so important?

TP53 encodes a transcription factor named p53, which has only 393 amino acids. It functions as a tetramer of two dimers, each binding a sequence RRRCWWGYYY (R = A/G, W = A/T, Y = C/T). When a gene contains two such sequences separated by 0–13 base pairs, it becomes a potential target of p53. Up to date, out of 30,000 human genes known so far, 3661 have been found to contain such p53 response elements. In another word, more than 10% of our entire genome is possibly under p53 regulation. Among these target candidates, 346 have been confirmed to be bound and regulated by p53, including 246 upregulated by p53, 91 downregulated by p53, and nine can go either way [31]. That is to say, p53 has the power to shut a gene down or open it up, "all up to its mood."

Normally, after translation, p53 is degraded rapidly through ubiquitination by MDM2, an E3 ubiquitin ligase that happens to be a true target gene of p53. In another word, p53 is a well self-disciplined molecule and can take good care of itself and would not allow itself to accumulate unnecessarily. In response to cellular stresses like DNA damage, oncogene activation, or hypoxia, however, p53 dissociates from MDM2 through various protein modifications such as phosphorylation, acetylation, or methylation, becoming an active transcription factor. Then, p53 rolls out a transcriptional program, namely activating certain genes and/or suppressing some others, to cause cell cycle arrest, senescence, or apoptosis, thereby managing the cellular crisis and bringing the microenvironment back to normal. For this reason, p53 has earned the honor as the "guardian of the genome," and also for this reason, a cell must depower p53 first in order to become malignant.

There are several ways to depower p53 in a cell. Gene mutation is the first one. As mentioned earlier, more than 50% of cancers have TP53 mutations. Interestingly, a majority of these mutations (~ 90%) are missense. In another word, the mutated gene can still be transcribed into a protein product, just different from the wild-type p53. Furthermore, most of these mutations take place at ~190 codons, which encoding the amino acid residues 102–292 within the DNA-binding domain of the transcription factor. Some of the mutant p53 protein products still possess DNA-binding capability to a degree, just weaker, about 0–75% of the wild-type p53 depending on the exact location of the mutation. This is also believed to be the reason for different cancerous phenotypes. Environmental carcinogens tend to cause selective mutations within TP53 and thereby lead to tissue-specific cancers. For instance, tobacco smoke (carcinogen: benzoapyrene diol epoxide) tends to induce mutations at G245 V, G245C, and R249M, which are commonly seen in association with ESCC patients [32]. *In vitro* studies have demonstrated that the expression of mutant p53 in normal cells with TP53 deletion gives them new properties like rapid proliferation, loss of contact inhibition, accelerated migration/invasion, and tumorigenic potential in nude mice, which are the properties that a cancer cell usually possesses, further indicating TP53 mutations in favor of cancer development.

Compared to gene mutation, posttranscriptional regulations also play a significant role in depowering p53. As discussed earlier, p53 protein is constantly degraded by MDM2-mediated ubiquitination. MDM4, a homolog of MDM2, can suppress p53 activation as well, and so do several others, like SIRT1, YY1, MTA2, and HDAC1. Cancer cells learn to cast curses on p53 by overexpressing these proteins in case TP53 mutation did not work. The expression of microRNAs is another example. Several species of microRNAs (i.e., miR-125b, miR-504, and

miR-25) have been found to directly bind to p53 mRNA and block it from translation and thereby allow cancer cell to proliferate. By the same principle, microRNAs targeting the suppressors of p53 can indirectly fight for p53 protein stability and activity. For instance, miR-192 increases p53 accumulation by targeting MDM2 mRNA; miR-191 supports p53 stability by blocking MDM4 translation, and miR-34a initiates attacks on YY1 mRNA.

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