REVIEW

Research on esophageal cancer: With personal perspectives from studies in China and Kenya

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Abstract

The most common form of esophageal cancer (EC), esophageal squamous cell carcinoma (ESCC), is prevalent in many unindustrialized societies, among people with lower socioeconomic status and those who frequently use tobacco and alcohol. In some areas, ESCC mortality ranked top among all cancer. In this review, we begin with discussions of the extensive research on EC in Linxian in northern China that started 60 years ago and the recent studies in Kenya from our personal perspectives. Based on the results obtained from these studies and information from the literature, we summarize our current understanding about the risk factors for ESCC including lifestyle factors (smoking, alcohol, consumption of food and beverages at high temperature and other unhealthy habits), poor diet and nutritional insufficiencies and genetic susceptibility. Elimination or minimization of these environmental risk factors, as well as early detection and treatment of precancerous lesions, would be effective means for the prevention of ESCC. Current knowledge of molecular alterations in ESCC (gene mutations, hypermethylation and amplification or overexpression), as well as treatment of ESCC and the potential of targeted therapy, are also discussed. Finally, we propose effective approaches for the prevention of ESCC by adapting a healthy lifestyle, including a healthy diet that would also prevent other diseases. Community outreach, public education and international collaboration are important for achieving this public health goal.

KEYWORDS
esophageal cancer, molecular alterations, prevention, risk factors, therapy

1 | INTRODUCTION

Esophageal cancer (EC) is one of the deadliest diseases in many parts of the world. There were an estimated 572 034 new cases and 508 585 cancer deaths in 2018, with almost half of new cases occurring in China.[1] The most common form, esophageal squamous cell carcinoma (ESCC), accounting for ~90% of all the cases of EC, occurs at high incidence in certain areas, such as the central Asia EC belt - starting from

the Taihang Mountain Range (TMR) in northern China to Central Asia and extending to Iran, and the African EC corridor in eastern and southern Africa. In Western countries, the incidence of ESCC has significantly declined in the past decades. Instead, another type of EC, esophageal adenocarcinoma, which is caused mainly by gastroesophageal reflux, has become the major form of EC.[2] This review article will focus on ESCC.

In China, most of the research activities on EC were historically centered in Linxian County (now named Linzhou City) of the Henan Province, located in the TMR. In this area, the EC mortality rate presented itself as concentric belts from a high incidence rate of >80/100 000 and descending to regions of >40, >20 and then <20/100 000. In August 1979, supported by a grant from the International Agency for Research on Cancer (IARC) and hosted by the Cancer Institute of the Chinese Academy of Medical Sciences (CICAMS) in Beijing, coauthor Chung S. Yang visited China to learn about EC research. Based on what he learned from Beijing and later in Linxian, Chung S. Yang wrote a review article titled "Research on Esophageal Cancer in China: A Review."[3]

The young ESCC cases reported by Dr Russell White in the 13th World Conference of OESO (World Organization for Specialized Studies on Diseases of the Esophagus) caught the attention of coauthor Xiaoxin Luke Chen. He wrote a review article on EC in Kenya[4] and visited Kenyatta National Hospital in Nairobi and Tenwek Hospital in Western Kenya in December 2018 to gather first-hand information on EC in Kenya.

In this article, we will first review the research activities in China and Kenya from the authors' personal perspectives. Then, based on available information in the literature, we will discuss our current understanding of the risk factors, molecular alterations, prevention and treatment of ESCC. Effective approaches for the prevention of ESCC and needs for future research will also be discussed.

2 | ESOPHAGEAL CANCER IN LINXIAN

2.1 | Early studies in Linxian

Linxian was a rural county with beautiful mountains, hard-working people and extensively cultivated land. Since the late 1950s, the EC problem had received attention from the Chinese central government. In 1959, many medical doctors and researchers from different areas were assigned to work on EC in Linxian. By 1979, much information on the risk factors had been accumulated, based primarily on observational epidemiology and information from the literature.[3] To avoid "eating moldy food," "using pond water" and "ingesting hot food and beverages" were promoted as measures for EC prevention. Moldy food could contain carcinogenic mycotoxins and precursors for N-nitrosamines, such as amines and amides. The nitrosating agent—nitrite could be derived from drinking water sources—usually from ponds. Nitrosamines, such as N-nitrosomethylbenzylamine (NMBA), can readily induce EC in rats[5] and were believed to be the leading candidate carcinogen of human EC.[3] A new nitrosamine, N-1-methylacetonyl-N-3-methylbutylnitrosamine, was found in moldy

cornbread at the level of 0.2 to 0.3 ppm.[6] Polyaromatic hydrocarbons (PAHs), generated from stoves near the bed (to keep warm in the winter), were also discussed as possible carcinogens for EC.[3]

A highly suspected moldy food was "pickled vegetables," which were commonly consumed in that area.[3] The presence of N-nitroso compounds in pickled vegetables and their mutagenic or cell transformation activity was later demonstrated.[7,8] The correlation between pickled vegetable consumption and EC mortality was observed in a large study in 1973.[3] However, some follow-up studies were said to be uninformative.

The importance of the treatment of precancerous lesions was recognized at that time. Esophagitis was found to be quite prevalent and was considered to be a precursor of EC by some scientists, but there was no real evidence that this condition itself leads to EC. Besides, there was no effective way of preventing or treating it, except "improve the eating habit." However, "early detection and early treatment" was well recognized. Of note was the outstanding work of Professor Qiong Shen who pioneered the use of balloon cytology in China for the screening, diagnosis and even localization of EC (Figure 1). Because of the economical strain, only some of the EC were treated by surgery (usually proficiently performed) or by chemotherapy or radiation therapy (not as advanced as the Western world). Balloon cytology also could pick up dysplastic cells (reflecting a precancerous state) occasionally, but there was no effective way of treating esophageal dysplasia at that time. Dr Shen was so dedicated to research on EC and moved to Linxian so he could be fully devoted to EC research. He once said, "We should try to solve people's problems with cheap and convenient means." This statement is still of great value to scientists and medical doctors now, especially with the current "for-profit" mindset.

Cigarette smoking and alcohol drinking were recognized as the major risk factors for ESCC in many parts of the world.[2] In Linxian, they were not because most people could not afford cigarettes or alcoholic beverages. A possible risk factor was the poor nutritional status of the population due to a monotonous diet with corn as the major staple. In 1979, peasants ate their share of grains and other food that were collectively produced locally. Commercial food items and refrigerators were not available. Consumption of meat, eggs, fish and dairy products was very low. The consumption of vegetables and fruits was low and seasonal and thus insufficiencies in micronutrients were suspected. People were lean; however, signs of overt nutritional deficiencies were rare. This encouraged us to pursue a biochemical approach to further assess the nutritional status of the population.[9]

In 1980, supported by a grant from the US National Academy of Sciences, Chung S. Yang worked in Linxian with a collaborative group from the Henan Tumor Institute and Henan Medical College to measure blood levels of ascorbic acid and carotenoids and accessed riboflavin nutritional status by the glutathione reductase assay. The study confirmed the suspected lower nutritional status or insufficiency of vitamin C, pro-vitamin A and riboflavin.[9] With these results, the idea of a nutritional intervention study was discussed among collaborators in Linxian, Zhengzhou and Beijing, and then with Dr William Blot of
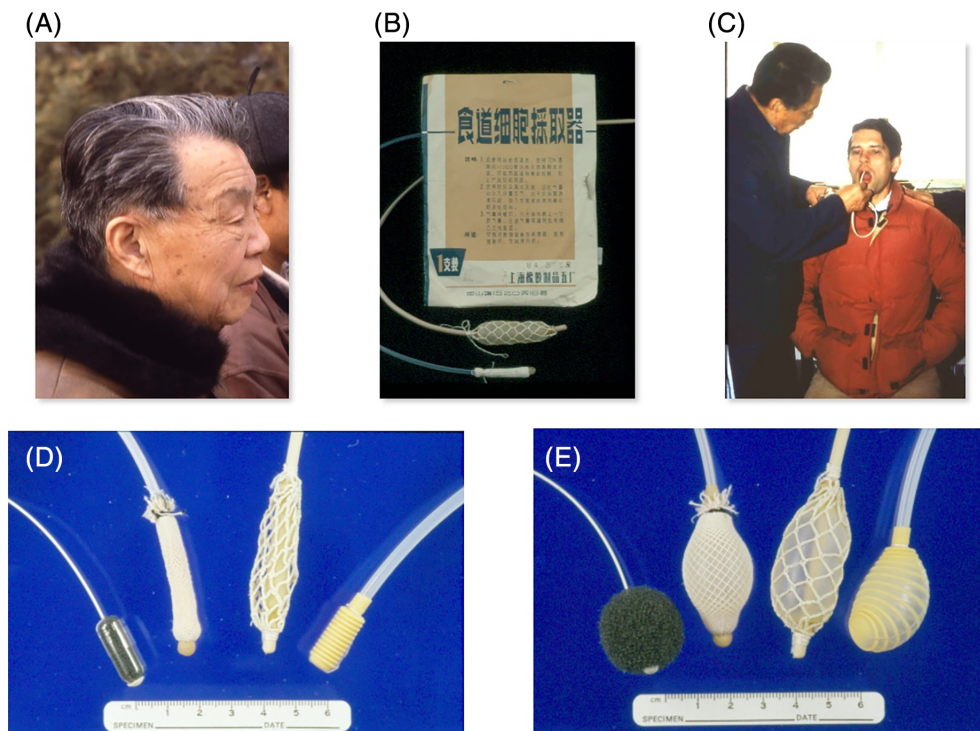
FIGURE 1 Professor Qiong Shen and balloon cytology. A, A photo of Professor Qiong Shen; B, balloons used in the 1970s for balloon cytology. C, Professor Shen performing balloon cytology on Dr Sanford Dawsey of the National Cancer Institute (NCI). D,E, Newer versions of the balloons used for cytology and a quotation from Dr Shen. (thank to Dr Dawsey for providing these photos)

Shen "Solve people's problems with cheap and convenient means"

the US National Cancer Institute (NCI). At that time, Dr Blot was visiting China and was interested in the idea. United States and China had recently established a formal diplomatic relationship, and the NCI was interested in establishing meaningful collaborative cancer research projects in China. In 1981, Dr Junyao Li, the chief epidemiologist from the CICAMS, was invited to the NCI to discuss US-China collaboration on cancer research. The US-China collaborative intervention studies would start a few years later.

2.2 | Nutritional interventional studies in Linxian

The hypothesis that supplementation with micronutrients would reduce EC risk in humans was tested in the 1980s in a large-scale intervention study on gastroesophageal cancer, the US-China cooperative Linxian Nutritional Intervention Trial (LNIT). This trial was conducted jointly by the CICAMS and NCI. NCI provided expertise in epidemiology and intervention trials as well as most of the funding. For example, Dr. Sanford Dawsey, providing expertise in pathology, worked meticulously with Chinese collaborators to establish the standard of diagnosis and conducted other key studies. CICAMS provided administrative support, medical doctors, pathologists and organized a large team of local paramedics to conduct the field work, including physical examinations, interviews for dietary and health information, delivery of intervention pills, compliance assessment and data entry (Figure 2). Chung S. Yang contributed his expertise in nutrition and cancer, conducted key studies and facilitated many of the research interactions. The advantages of conducting a nutritional intervention

trial in Linxian were (a) the high mortality rates of EC, especially in the northern part of Linxian; (b) the known nutritional insufficiencies and stable dietary pattern; (c) the stable population—a person can be found either at home or in the field; and (d) the well-structured society—people followed the instructions from authorities.

After many planning meetings and several site visits to Linxian starting in 1982, the study was launched in 1985. Because many micronutrients had been implicated in gastroesophageal cancer, there were many different opinions on what nutrients to use. Finally, a compromise was made that nine nutrients were grouped into four sets (factors): (A) retinol and zinc; (B) riboflavin and niacin; (C) ascorbate and molybdenum; and (D) α-tocopherol (α-T), β-carotene, and selenium (as selenium yeast), with each nutrient at 2 to 3 times the dose of the US Recommended Daily Allowance. These Sets of nutrients were combined in a factorial design in eight groups: placebo, AB, AC, AD, BC, BD, CD and ABCD. The study involved 29 584 adults (aged 40-69) who were given daily supplementation as pills for 63 months (1985-1991). The CICAMS established a fieldwork team stationed in Linxian and the US collaborators routinely visited Linxian, at least once or twice a year, to check the progress and provide expertise in different areas. During the trial, blood samples from some of the subjects were collected for analysis of compliance and for establishing a serum bank for future nested case-control studies.

There were 2127 deaths during the trial period; 32% were due to ESCC and gastric cancer—mainly gastric cardia cancer (GCC). Gastric cardia is located just below the esophagus and GCC was also considered as "esophageal cancer" by residents in Linxian, where GCC accounted for 40% of the "esophageal cancer" and the other 60%

FIGURE 2 Photos of the Linxian Nutritional Intervention Trials. A, Site visit of National Cancer Institute (NCI) and other investigators to Linxian before launching the intervention. B,C, Interviewing and physical examination at the beginning of the trial. (Photos taken by Chung S. Yang.) D, Chung S. Yang (in striped shirt) observing pill counting for compliance analysis

was ESCC. Factor D, a combination of α-T (50 mg), β-carotene (15 mg), and selenium (50 μg), was found to decrease mortality from gastric cancer (mainly GCC) by 20% and total cancer mortality by 13%, while the ESCC mortality was not affected.[10] Other nutrient combinations in Factor A, B or C, however, did not show any significant effect on the endpoints measured. Subsequent nested case-control studies showed that the baseline serum levels of α-T and selenium were low, and were each inversely associated with gastroesophageal cancer risk.[11,12] The result is consistent with the concept that the low antioxidant (vitamin E and selenium) nutritional status makes the individuals susceptible to inflammation and carcinogenesis.[13] Under these conditions, supplementation with vitamin E (α-T) and selenium attenuated carcinogenesis.

Furthermore, results from a 10-year follow-up showed that the preventive effect of the combination of α-T/β-carotene/selenium on gastric cancer and total mortality was sustained and that this nutrient combination also protected against ESCC in younger subjects—when they were enrolled in the trial at ages 55 or younger, but not in those older than 55.[14] It is possible that the intervention was ineffective in older subjects because they already had higher-grade precancerous lesions. This concept is consistent with the result of a parallel trial in Linxian on subjects with esophageal dysplasia, showing a lack of beneficial effect by supplementation with a combination of micronutrients.[15] In the 25-year post-trial follow-up of the LNIT, the previously observed protective effects of α-T/selenium/β-carotene

were attenuated or lost.[16] However, an apparent new protective effect against ESCC was found for Factor B (riboflavin and niacin).

The above cancer-preventive effect of α-T/selenium is supported by studies in a rat model demonstrating that a diet with insufficiencies in α-T/selenium enhanced NMBA-induced esophageal carcinogenesis, and that supplementation of α-T/selenium at the early stage of the experiment was more effective than that at the late stage.[17] These experiments demonstrate the cancer-preventive activity of antioxidant nutrients (α-T and selenium) when supplemented to a human population or rats with low antioxidant nutritional status.

2.3 | Socioeconomic development and lowered EC risk in Linzhou

Living in the mountain range, the men in Linxian (the old name) were traditionally skilled at stonework and in modern days, the skill has been transformed into construction work. During the past 40 years, there has been tremendous construction work in China and the construction teams from Linzhou worked all over the country. This resulted in the accumulation of capital in Linzhou and helped the rapid socioeconomic development in Linzhou; the GDP per capita increased from ¥186 in 1978 to ¥68 501 in 2017 according to data from the National Bureau of Statistics of China. When Chung S. Yang revisited Linzhou City in 2019, the city was well developed with many shops

and restaurants. This urbanization was in sharp contrast to 40 years ago.

The Linzhou Cancer Hospital is now a modern hospital in a big high-rise building. In the conference room of the hospital, Vice Director Dr Zhicai Liu showed that the age-adjusted incidence rate of EC (including GCC) for males had decreased from approximately 110/100 000 to about 55/100 000 from 1988 to 2013. The age-adjusted death rate was slightly lower and showed a similar decrease. The incidence and death rates for females were significantly lower than men and also showed a similar decrease by about 50% from 1988 to 2013. Apparently, the improvement of diet, nutrition, quality of water and oral hygiene due to economic development had contributed to the decrease of EC in Linxian and some other areas, but such an extensive decrease has not occurred in some economically less developed areas in China.[18-20] Ironically, the increased consumption of alcohol and cigarettes in Linzhou and other areas nowadays is expected to compromise the beneficial effect of economic development on EC.

Of note is that during the past 40 years, especially after 2000, the overall cancer rate has markedly increased in Linzhou—among them cancers of the lung, breast, ovary, liver and the colorectum. The rate of cardiovascular diseases also increased. These follow the same disease pattern of China as a country,[21] possibly due to economic development and commercialization; the consumption of meat, fat, refined grain and total calories as well as cigarette and alcoholic drinks had increased, while physical activities had decreased. Does this mean we expel one devil and invite another? A recent 26-year follow-up study of LNIT based on food frequency questionnaires in 1984-1991 found that higher intake of grains, vegetables, beans, fruits and nuts was associated with a lower risk of gastric (cardia) cancer, heart disease and stroke mortality.[22] Apparently, the best approach to prevent EC is to adapt a healthy lifestyle, including a healthy diet, which will also reduce the risk of other chronic diseases.

3 | STUDIES ON EC IN KENYA

EC (mostly ESCC) is ranked the third in its incidence among all cancers in Kenya. It ranked second after prostate cancer in men and third after breast cancer and cervical cancer in women. Yet, EC is the number one cancer killer in Kenya. In 2018, among 33 978 cancer deaths, 4351 (16%) were due to EC.[23]

ESCC in Kenya shares many features of ESCC in China. The proportion of Kenyans living below the international poverty line was 46.8% in 2005/2006 and 36.1% in 2015/2016. Kenya belongs to a high incidence region known as the African EC corridor Rift Valley in Western and Central Kenya, similar to the North Central TMR in China. Kenya shares many recognized risk factors of ESCC, which include tobacco smoking, alcohol drinking, scalding hot food or drink, deficiency of micronutrients, food and drink containing carcinogens, familial history of cancer—especially ESCC.[2,4] Liquor (*Chang'aa*, containing >50% alcohol) is a popular social drink in rural Western Kenya where binge drinking is a very common practice. A recent

epidemiological study showed that alcohol consumption, particularly of *Chang'aa*, contributes to half of the ESCC burden in western Kenya.[24] Human papilloma virus infection is not likely associated with the ESCC in Kenya, nor in other parts of the world.[25] In Kenya, 70% to 80% of cancer cases are diagnosed in late stages, and only a few hospitals treat EC patients. Self-expandable metallic stents applied under endoscopy are the most frequently used form of palliative treatment.

Nevertheless, ESCC in Kenya has some unique features. NextGen sequencing (NGS) of human ESCC samples from Malawi (a neighboring country on the African EC corridor) identified a unique mutation signature characterized by $C \rightarrow A$ transversion and $C \rightarrow T$ transition, suggesting a unique etiology of ESCC in this region.[26] A large-scale sequencing project supported by Cancer Research UK (Mutographs of Cancer) may provide important insights into the etiology.

3.1 | Young EC

ESCC is rare among young people (<30 years) in most countries, 0.7% in northern China, 1% in northeastern Iran and 0.5% in the United States. Most ESCC patients are diagnosed at the age of >50 with an average of ~65 years old. However, in Kenya, one study in 2010 from Tenwek Hospital reported 6.3% (58/914 cases) occurred in patients <30 and 1% (9/914 cases) in patients <20.[27] An earlier report in 2002 from the same hospital reported even 11% (26/227 cases) of ESCC patients aged <30, and 2% (4/227 cases) aged <20.[28] The youngest case was at the age of 8 in this hospital. Unfortunately, ESCC in many young patients has already progressed to the late stage and long-term survival is rare.[29]

3.2 | Kalenjin ethnicity

Kenya has 42 ethnic groups among which the Kalenjin (~12% of the population) have a strikingly high incidence of young ESCC. The Kalenjin constitute the largest community residing in the northern and southern parts of Rift Valley. The main economic activity in the region is farming. According to a study from Tenwek Hospital, the proportions of young ESCC among Kalenjin cases and non-Kalenjin cases within the catchment area were 8.8% and 3.9%, respectively, and the proportions of young ESCC among Kalenjins and non-Kalenjins outside the catchment area were 11.1% and 1.1%. Young ESCC was strongly associated with Kalenjin ethnicity, but not with a residency in the catchment area. However, being Kalenjin was not associated with an increased risk of ESCC if cases of all ages were pooled together.[27] Kalenjins may have unique genetic makeup and they are well known to produce many top marathon athletes. At Tenwek Hospital, in 60 cases of young ESCC, tobacco smoking was only observed in 15% (9/60) and alcohol drinking in 15% (9/60). Whereas among 49 young ESCC cases with family history data, 21 cases (43%) had a family history of ESCC, and 5 cases had multiple cases in the family. These data

suggest that genetic factors and shared environmental factors, but not smoking and drinking, are important in causing ESCC in Kalenjins, in particular, young Kalenjins.[29]

3.3 | Mursik consumption

Fermented milk (*Mursik*) is a traditional and popular drink in Kenya. Many Kenyans do not like the flavor of fresh milk and ~80% Africans cannot tolerate milk due to lactose intolerance.[30] Every tribe has its own unique method of production. For example, in Kalenjin tribe, the inside of calabash gourds is first coated with charcoal dust. Whole cow milk is boiled, poured into the gourd, mixed with left-over fermented milk (as a starter) and left for fermentation for several days at room temperature. Small quantities of cow blood may be added as a source of iron to facilitate microbial metabolism. Sometimes mursik may be stored for over a year before consumption. Refrigerators are not available in most of these households.

Most mursik starter culture collected from Kalenjin families contained mutagenic levels of acetaldehyde (>100 μM; sometimes >1000 μM) in addition to >100 mM of ethanol and potentially PAHs from the charcoal.[31] Regular consumption of mursik by young children may contribute to young ESCC in Kalenjins. According to a survey of 383 Kalenjin families in 2013, 32% of households fed their children (<5 years) with mursik with a mean quantity of 250 mL per week.[32] There is an incentive to let the children consume more mursik due to nutritional needs and a lack of other dairy products. It is well known that acetaldehyde induces nasal and respiratory tract squamous cell carcinoma in rats and hamsters when inhaled.[33,34] Mechanistically, as a highly reactive compound, acetaldehyde attacks nucleic acids and amino acids and causes DNA mutations and protein dysfunction.[35-37] However, it remains unclear how acetaldehyde exposure may cause ESCC in humans.

3.4 | Scalding hot tea drinking

Tea (*chai*) drinking is part of the Kenyan lifestyle. Black tea with milk is usually served right away from a boiling tea kettle 2-3 times every day. At Tenwek Hospital, a recent study showed that 99% (99/100) of local healthy participants preferred tea at the temperature of >65°C, a temperature classified by IARC as possibly carcinogenic (Group 2A) to the esophagus.[38] In Western Kenya, ESCC cases were 12.3 times more likely to drink scalding hot food compared to the controls.[39] It is known that hot water (65°C) significantly increased the number of tumors and the incidence of ESCC in NMBA-exposed rats.[40]

3.5 | Khat chewing

Chewing khat (*Catha edulis*) leaves for the pleasurable stimulant effect is a widespread habit in East Africa. Khat is one of the most popular and lucrative, yet legal, drugs in Kenya. Other than its systemic effects, chronic khat chewing has been associated with periodontitis, leukoplakia, oral cancer, esophagitis and EC,[41-44] similar to betel quid (Group 2A) chewing in other regions.

3.6 | Cultural taboo

In rural Kenya, most ESCC patients came for medical care at the late stage. In the surrounding communities that Tenwek Hospital serves, there is a false conception that medical treatment by surgery or esophageal stent causes death in months. Herbal therapy is often believed as the best treatment option. Patients are fear of being diagnosed and feel shameful in seeking medical care because EC is thought to be contagious. Thus, delay in hospital visits or diagnosis is a common problem.

3.7 | Poverty

Lower socioeconomic status has long been associated with higher risk and mortality of ESCC according to many studies in different parts of the world including China.[18-20] Such health disparity can be expressed in sex differences, racial and ethnic differences and urban-rural differences. In fact, poverty explains many risk factors of ESCC in Kenya.

4 | RISK FACTORS AND PREVENTION OF ESCC: WORLD PERSPECTIVE

Numerous reports indicate that environmental and lifestyle factors, which are related to lower socioeconomic status, are the major causes of ESCC worldwide.[2,3,18-20] However, the relative importance of these factors may vary in different populations. In the Western world, tobacco and alcohol are more important risk factors for ESCC than in most high-incidence areas in the developing countries. For example, in a recent cohort study in northeastern Iran, a 10-year follow-up showed that 76% of EC was attributable to the combination of the following risk factors: unpiped water, opium smoking, tooth loss, low intake of vegetables and fruits, hot tea and indoor pollution.[45] Because of the economic development, the diet may improve, but smoking and drinking may become more prevalent; the relative contribution of smoking and drinking to ESCC is expected to increase. These and other important etiological factors are summarized as follows.

4.1 | Tobacco, alcohol and other unhealthy substances

Tobacco smoking and chewing are recognized as major risk factors for ESCC in many areas of the world.[2,46-48] The risk for ESCC is usually threefold to ninefold higher in current smokers compared to non-smokers. Tobacco and tobacco smoke contain numerous carcinogens

such as nitrosamines, PAHs and reactive aldehydes—existed in tobacco or produced during smoking.

Alcoholic beverage consumption has consistently been found to increase the risk of ESCC in most parts of the world, and IARC has causally linked alcohol to ESCC.[2] Alcohol is metabolically converted to acetaldehyde, which is recognized as a class I carcinogen.[36-38,49] Genetic polymorphisms associated with lower ALDH activity are associated with increased risk of ESCC.[50] Some alcoholic beverages and fermented foods also contain acetaldehyde. Tobacco and alcohol may interact synergistically to increase the risk of ESCC,[51] partially due to ethanol enhanced absorption of carcinogens by epithelial cells.

Betel quid chewing used to be common in the south and southeast Asian countries, such as India and Taiwan.[52] In Taiwan, lime (calcium hydroxide) was added, and chewing was said to produce stimulants. Upon chewing the nuts in the presence of lime, the polyphenolic compounds from betel are rapidly oxidized to produce reactive oxygen species that damage oral and esophageal epithelial cells to induce mutations and cancer. The IARC has confirmed betel quid as a risk factor for ESCC and betel chewing and smoking may have a synergistic effect to induce ESCC.[52] The association of khat chewing and opium smoking (produces PAHs) with ESCC was found in Kenya and Iran, respectively.

4.2 | Dietary and nutritional factors

Studies in the TMR areas in northern China, Iran, South Africa and other areas indicated that ESCC was associated with a poor monotonous diet. The consumption of vegetables and fruits, legumes, meat and dairy products was usually low. Nutritional insufficiencies are highly suspected to be linked to ESCC.[10-12,53-57] Insufficiency of folic acid, which affects one-carbon metabolism and may increase the promoter hypermethylation leading to the silencing of many tumor suppressors and receptors, may also be a contributing factor to ESCC, especially in individuals with low methylenetetrahydrofolate reductase activity.[58] Other micronutrient insufficiencies may also increase the risk for ESCC and other cancers, but the evidence is not as strong as that shown in the LNIT.[57,59]

Even though mycotoxins and *N*-nitroso compounds are highly suspected as carcinogens for ESCC,[3] strong evidence in humans is still lacking. The pickled vegetables from Linxian were shown or suspected to contain these types of carcinogens; however, there was limited evidence in humans to support the carcinogenicity of pickled vegetables.[60]

4.3 | Other risk factors

Eating foods and beverages at a high temperature has been demonstrated to be an etiological factor for oral cancer and EC. This includes studies in China, Kenya and Iran, and the temperature may exceed 70°C.[38,45,61,62] Hot temperature can cause thermal irritation of the esophageal epithelium, which may promote inflammation and

enhance the action of chemical carcinogenesis in animal models.[63] The high incidence of EC in South America due to the consumption of hot maté may be due to the combined action of the high temperature and the carcinogenic PAHs in maté.[64,65]

Early studies in Linxian suspected that poor oral hygiene leading to tooth loss might cause the individual to chew hard food insufficiently and irritate the esophageal mucosa. The association between tooth loss and EC was confirmed in a prospective study in Linxian.[66] Regular tooth brushing has been reported to be associated with reduced risk of ESCC in many studies including those conducted in China, Iran, Kenya and India.[2] A possible mechanism for the association is that poor oral hygiene could enhance inflammation associated with dental disease or affect oral microbiota.[2,67]

4.4 | Genetic susceptibility

Since 2010, there have been several papers on genome-wide significant association study (GWAS) based on the analysis of single nucleotide polymorphism (SNP) assessing the possible association between high-prevalence but low-penetrance genetic variations with EC in China. Susceptibility loci at 10q23 associated with phospholipase c epsilon 1 (*PLCE1*), was found for both ESCC and GCC by two research groups, independently.[68,69] The functional importance of *PLCE1* has been elucidated.[70] In rodent models, knockout of *PLCE1* decreased susceptibility to chemically or genetically induced ESCC, skin or colon cancer.[71-73] Other studies and joint analyses of data from different GWAS revealed many other susceptibility loci.[74-77] Some of these variants, with low frequency or low risk association, may only have a limited role in ESCC. In combination with environmental factors, however, some of the genetic polymorphisms could play an important role. For example, variants at the 4q23 locus, which includes the alcohol dehydrogenase (ADH) cluster, each had a significant interaction with alcohol drinking in their association with ESCC risk. The known association of the aldehyde dehydrogenase 2 (ALDH2) locus on 12q22 with ESCC[50] was confirmed, and drinkers with both the *ADH1B* and *ALDH2* risk alleles had a fourfold increased risk for ESCC in comparison to drinkers without these risk alleles.[75] An rs138478634-GG phenotype, most likely due to a higher capacity of variant CYP26B1 to catabolites trans-retinoic acid, a possible anti-cancer nutrient, enhanced the risk for developing ESCC.[78] The risk may be further increased in individuals with low vitamin A nutritional status. More studies on the genetic susceptibility or heritability of ESCC are needed.

4.5 | Prevention of EC—Healthy lifestyle and screening for precancerous lesions

We propose three approaches for the prevention of ESCC: (a) Remove or avoid risk factors, such as tobacco, betel quid, khat, opium, food that may contain carcinogens, excessive alcohol, hot food and beverages and poor oral hygiene. (b) Eat a healthy diet that

provides sufficient nutrients. This is especially true for the populations in the high EC incidence areas, where consumption of vegetables and fruits are low. (c) Early detection and treatment can reduce the incidence and mortality of ESCC, as demonstrated in several screening studies in China. For example, in an endoscopic screen study of ESCC in Linzhou, residents age 40 to 69 years were recruited from communities with high rates of ESCC and 3319 volunteers were screened in the intervention group during 2000 to 2001. Those with dysplasia or occult cancer were treated. During the 10-year follow-up, 652 incident cases and 542 fatal ESCC were identified. The cumulative incidence and mortality in the intervention group (4.17% and 3.35%, respectively) were significantly lower than the control group (5.92% and 5.05%, respectively), which consisted of residents who did not receive the endoscopic screening in villages that were geographically adjacent to the intervention villages.[79]

With economic development in China, endoscopic examination becomes affordable and endoscopic screening in a high incidence area is a practical approach. The National Cohort of Esophageal Cancer-Prospective Cohort Study of Esophageal Cancer and Precancerous Lesions based on High-Risk Populations (NCEC-HRP), an open-ended prospective cohort study of screening supported by the National Key R&D Program, was launched in eight areas around China in June 2017 and a total of 100 000 participants will be enrolled by December 2020; the screening protocol was published.[80] In a recently published large population-based prospective study, 21 111 participants aged 40 to 69 years from three high-risk areas in China were endoscopic screened in 2005 to 2009 and followed through 2016. Increasing grade of esophageal squamous dysplasia was found to be associated with increased risk for ESCC incidence and mortality. The study suggests that in high-risk areas in China, dysplasia/carcinoma in situ and moderate dysplasia should receive therapy. The first screening should start at age 50 years old, and endoscopic surveillance intervals for moderate and mild dysplasia should take place in 3 and 5 years, respectively.[81]

5 | RESEARCH ON MOLECULAR ALTERATIONS AND TREATMENT OF ESCC

5.1 | Early studies on molecular alterations

In the 1990s, in collaboration with Dr Lidong Wang of Henan Medical University, Chung S. Yang studied the molecular events in the genesis of ESCC using resected samples or biopsies obtained from hospitals in Linxian and nearby areas. We found TP53 mutation was a common and early event, occurring even in precancerous lesions.[82-85] Loss of heterozygosity of the tumor suppressor Rb gene, correlated with loss of Rb protein expression, was also a frequent event.[86,87] Hypermethylation was found to be a mechanism for silencing the expression of HGMT, RARβ, p15, p14, hMLH1, FHIT and HLA Class I genes and other genes.[88-91] It is interesting that during the progression of carcinogenesis, generally the frequency of hypermethylation of a particular gene increased and additional genes became hypermethylated. We hypothesized that in combination with TP53

mutation, the promoter hypermethylation profile of genes could be used as biomarkers of the progression of ESCC and would be useful to identify precancerous lesions and predict the development and progression of ESCC. However, we were unable to fully test this hypothesis.

5.2 | Mutations and other genetic changes

With the advancement of the high-throughput sequencing technology, large-scale comprehensive analyses of ESCC have been conducted. Ohashi et al[92] summarized four large studies on ESCC in China. The most frequently mutated gene was TP53 (59%-93%), confirming the results of many previous studies, and suggesting its important role in the development of ESCC. Mutation of genes that are involved in cell cycle regulation (CDKN2A, RB1, NFE2L2, CHEK1 and CHEK2) or cell differentiation (NOTCH1 and NOTCH3) have been detected at frequencies of 2% to 10%. Many cell cycle regulating genes were also amplified (CCND1 at 46.4%, CDK4-CDK6 at 23.6% and MDM2 at 5.7%). EGFR overexpression in ESCC is often accompanied with amplifications in downstream signaling molecules, such as RAS and AKT pathways.[92] A more recent comprehensive molecular analysis of 164 ESCC samples found frequent genomic amplifications of CCND1, SOX2 and TP63.[93]

Up to now, more than a dozen of NGS studies have been performed on ESCC from China and elsewhere, generated a lot of information on genetic alterations and shed light on etiology, carcinogenesis and therapeutic targets.[94] In addition to a list of genetic and epigenetic alterations as briefly described above, there is a much in-depth understanding of molecular subtyping,[93] racial differences,[93,95] clonal evolution from precancerous lesions,[96,97] genetic heterogeneity and lymphatic metastasis.[98,99] Based on these studies, a list of potential therapeutic targets has been proposed for further studies: for example, receptor tyrosine kinases (EGFR, FGFR1, PI3K/AKT), nuclear factor erythroid-derived 2 (NRF2) pathway, squamous differentiation mechanism (SOX2-TP63, NOTCH), cell cycle control (TP53), WNT pathway, Hippo pathway, nuclear exportin (XPO1) and DNA repair pathway (BRCA1).

Of note is that somatic mutations of cancer-related genes do not necessarily lead to the development of ESCC. Several groups discovered that histologically normal esophagus has a relatively higher mutation burden than most other tissues. The mutation burden was associated with both age and cell proliferation rate, suggesting that mutations in the esophagus accumulate over time with cell division.[100] Mutant clones can appear in early childhood, then increase in number and size with aging, and ultimately replace larger areas of the esophageal epithelium in the elderly. Exposure to environmental factors, heavy smoking and drinking, further speeds up this process.[101] Surprisingly, the prevalence of NOTCH1 mutations in the histologically normal esophagus was much higher than that in ESCC. It was suggested that the expansion of multiple NOTCH1-mutated clones made epithelium prone to carcinogenesis by exposure to tobacco and alcohol.[101,102]

5.3 | Therapy for ESCC

While esophagectomy remains the cornerstone, endoscopic procedures for early lesions, neoadjuvant chemoradiotherapy and minimally invasive esophagectomy have been successfully and widely used for clinical management of ESCC.[103-105] However, early lymphatic metastasis in some cases remains a challenge and makes outcomes unpredictable. Patient-derived cell lines, xenograft models, and three-dimensional organoids have been tested for selecting sensitive chemotherapeutic agents and determining drug resistance before treatment.[106-108] For the clinical follow-up, circulating tumor DNA analysis has emerged as a potentially valuable tool when used together with existing tools.[109]

In the era of personalized medicine, targeted therapy is a very promising treatment modality when used in combination with other therapies.[110,111] Anti-PDL1 immunotherapy with pembrolizumab (Keytruda) has been approved by US FDA for locally advanced or metastatic ESCC that has progressed after treatment with one or more lines of standard therapy. In patients with high levels of PD-L1, a response rate of ~20% was achieved in two clinical trials.[112,113] Anti-EGFR therapy failed to show much clinical benefit, although EGFR overexpression is commonly seen in ESCC.[114]

Apart from these molecular targets shared with other cancers, some other targets are relatively specific for ESCC and require extensive basic research to develop corresponding drugs. Xiaoxin Luke Chen and associates have focused on NRF2 as a drug target for ESCC with NRF2 hyperactivation.[115] As a major cellular defense mechanism, the NRF2 signaling pathway is known to regulate the expression of antioxidant and detoxification enzymes. However, hyperactive NRF2 also helps cancer cells survive chemoradiation-induced oxidative stress and accelerates drug metabolism thus contributing to chemoradioresistance and poor prognosis, as well as metabolic reprogramming.[116,117] Several NRF2 inhibitors have been identified through high-throughput screening and these inhibitors have shown promising in vitro and in vivo efficacy in preclinical models.

6 | CONCLUDING REMARKS

During the past 40 years, much has been learned about the etiology and molecular alterations in the development of ESCC associated with lifestyle and nutrition. These would help us design strategies for the prevention, early detection, diagnosis and treatment of EC. From a socioeconomic point of view, a public health approach in the prevention of EC is of fundamental importance, and it requires planning and commitment from the government and the people. Within the research community, a multidisciplinary approach is needed to unite all relevant specialties to deliver cost-effective strategies against EC.

6.1 | Healthy lifestyle and healthy diet

It is important to adopt a healthy lifestyle by avoiding the use of tobacco and other unhealthy substances, limiting the use of alcohol,

maintaining good oral health and avoiding the consumption of hot food and beverages. Of equal importance is to eat a healthy diet that would provide sufficient nutrients, including eating plenty of vegetables and fruits. All of these depend on socioeconomic development so people can afford the needed food and have the information and household appliances to make healthy choices. As we learned from the example in Linzhou and other areas, as the economy developed, even though the occurrence of EC decreased, many other cancers (such as lung, colon and breast cancers), cardiovascular diseases and other diseases increased, possibly due to the increased consumption of cigarette, alcohol, fat, meat and sugar as well as reduced physical activity. Therefore, healthy lifestyle and healthy diet should be consistently emphasized.

6.2 | Community outreach

Studies in China have proved that extensive community outreach is the essential foundation for the fight against EC. Health education through the education system, community activities and news media will be essential in delivering accurate and useful information on EC, avoiding risk factors, and recruiting subjects for screening and clinical trials.

6.3 | Future research

Even with the many important discoveries in mechanisms and therapy on EC, many research questions remain unanswered and require extensive research. Epidemiological research has suggested modifiable risk factors; high-quality research epidemiological research is still needed in developing countries to better clarify the etiology of EC. How to reduce cancer risk based on this information through intervention remains a big challenge and needs more research. GWAS has identified SNPs significantly associated with cancer risk; the functional roles of these SNPs and their interactions with environmental factors remain to be further elucidated. NGS has revealed potential drug targets and much research efforts are needed for developing targeted therapy. Further research is needed to increase our understanding of the development, biochemistry and molecular biology of the esophageal squamous epithelium and to develop effective means for drug screening.

6.4 | International collaboration

International collaboration is essential for the progress of EC research. This is illustrated by the extensive collaboration between China and United States during the past decades.[118] These collaborations greatly enhanced our understanding of EC from prevention to treatment and helped train many researchers. It is encouraging that lately an African Esophageal Cancer Consortium was formed to help Africa in fighting EC.[119] OESO has organized a global education platform in

esophagology to help developing countries and share experiences globally. More such collaborations are needed.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

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