Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-1290

Reviewer A

The authors investigated the prevalence and prognostic role of SOX2 amplification and expression in surgically resected esophageal squamous cell carcinoma (ESCC). SOX2 amplification is an independent poorer prognostic factor, but chromosome 3 gain is an independent favorable prognostic factor. It seems to be better to research the relationship between SOX2 amplification and chromosome 3 gain in vitro study.
Reply: Thank you for pointing out this. That's what we want to do in the future.

Comment 1: Is there any relation between SOX2 amplification and chromosome 3 gain in each individual case?
Reply 1: In our study, 20 (4.4%) cases was SOX2 amplification, which met the two criteria: 1) ≥4 red target signals in no less than 30% tumor cells; 2) less than three green signals. Fifty-eight (12.9%) cases were defined as chromosome 3 gain with three or more green signals. Therefore, there was no relation between 4.4% cases with SOX2 amplification and 12.9% cases with chromosome 3 gain. Sorry for our misleading explanation. The sentence was revised as follows.

Changes in the text: in Line 1, Association between gene copy number variation and clinicopathological characteristics, Results, "Among 450 patients, 20 (4.4%) cases met the inclusion criteria (≥4 red target signals in no less than 30% tumor cells and less than three green signals), and these cases were classified as patients with *SOX2* amplification."

Comment 2: What do the authors explain that SOX2 amplification is an independent poorer prognostic factor, while that chromosome 3 gain is an independent favorable prognostic factor.
Reply 2: Just as what we said in discussion, it is possible that there are multiple targets for chromosome 3 gain and co-amplification of adjacent oncogenes can have a synergistic effect. Other genes reported to be putative targets in chromosome 3 include PIK3CA, SKIL, TERC, DCUN1D1, TP63, and EVI1. Therefore, the division of SOX2 amplification and chromosome 3 gain was reasonable.

Reviewer B

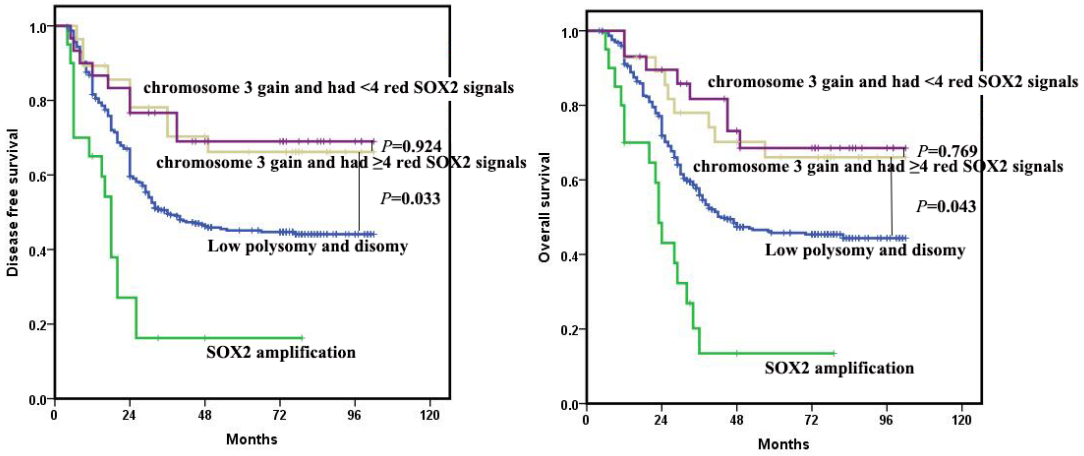
In this study, the authors investigated SOX2 amplification and prognostic role in esophageal squamous cell carcinoma (ESCC). They examined 474 ESCC by FISH and 4.2% ESCC were found with SOX2 amplification, and 12.4% with chromosome gain. SOX2 amplification is associated with later clinical stage, and 3q chromosome gain is correlated with the early clinical stage. SOX2 expression is significantly associated with copy number variations. SOX2 amplification showed poorer prognosis, and it was proved to be an independent prognostic factor. Chromosome 3q gain showed a better prognosis. These findings suggested that SOX2/3q chromosome genomic status may be indicative for the prognosis of ESCC. This finding is interesting, however, there are many concerns for publication.

Major

1. Are 3q chromosome gain and SOX2 amplification exclusive?

Reply 1: In our study, 20 (4.4%) cases were SOX2 amplification, which met the two criteria: 1) ≥4 red target signals in no less than 30% tumor cells; 2) less than three green signals. Fifty-eight (12.9%) cases were defined as chromosome 3 gain with three or more green signals. Therefore, there was no relation between 4.4% cases with SOX2 amplification and 12.9% cases with chromosome 3 gain. Sorry for our misleading explanation. The sentence was revised as follow.

Also, among 58 cases were with chromosome 3 gain, 28 (6.2%) case had ≥4 red SOX2 signals in more than 30% tumor cells, whose survival were similar to the other 30 (58-28) cases with chromosome 3 gain (Figure).



Changes in the text: In Line 1, Association between gene copy number variation and clinicopathological characteristics, Results, "Among 450 patients, 20 (4.4%) cases met the inclusion criteria (≥4 red target signals in no less than 30% tumor cells and less than

three green signals), and these cases were classified as patients with *SOX2* amplification."

2. The author described that the survival rate is 15-25% of ESCC in the discussion session (reference 2), however it was reported to be 50% in resectable advanced ESCC. Please clarify the prognosis of ESCC accurately. In my institute, for example, resectable advanced ESCC showed 70% survival rate. Is your case limited to unresectable advanced ESCC?

Reply 2: As we all know, there are differences in mortality rates among countries. Therefore, the reference 2 was revised as reference based on Chinese data [Changing cancer survival in China during 2003-15: a pooled analysis of 17 population-based cancer registries.]. The sentence was revised as follows.

In our hospital, the 5-year DFS and disease-specific OS rates for resectable advanced ESCC were 46.7% and 47.2%, respectively. Our result is consistent with other studies. For example, a study from National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, the 5-year OS rates is 47.08% in ESCC patients receiving radical esophagectomy [Nomogram to Predict Overall Survival for Thoracic Esophageal Squamous Cell Carcinoma Patients After Radical Esophagectomy, Ann Surg Oncol. 2019 Sep;26(9):2890-2898]. In a study with 1,220 ESCC patients who underwent complete resection from Guangdong Esophageal Cancer Institute, Guangzhou, Guangdong, People's Republic of China, the 5-year survival rate was 48.2 % [The Impact of Tumor Cell Differentiation on Survival of Patients with Resectable Esophageal Squamous Cell Carcinomas, Ann Surg Oncol. 2015 Mar;22(3):1008-14].

Changes in the text: In Line 5, Introduction, "Despite optimization of surgery, radiotherapy, and cytotoxic chemotherapy, survival of advanced ESCC is poor, with the age-standardised 5-year relative survival rate of only 30%(2)."

Reference 2 was revised as "Zeng H, Chen W, Zheng R et al. Changing cancer survival in china during 2003-15: A pooled analysis of 17 population-based cancer registries. The Lancet. Global health 2018;6;e555-e567."

3. SOX2 gene amplification is 40% in NGS of TCGA. This is totally different from your result. Please explain this large discrepancy.

Reply 3: In Lin' study, the amplification rate is 10% in ESCC [Genomic and molecular characterization of esophageal squamous cell carcinoma, Nat Genet. 2014 May ; 46(5): 467–473]. That's to say, there was large discrepancy between different studies, even though with the same detection method (NGS). In our study, fluorescence in situ hybridization (FISH) was used to detect SOX2 gene amplification. FISH has been

widely used in detecting specific gene copy number abnormalities in a variety of neoplasias. The advantages of FISH methods are as follows: 1) with the visualization of individual cells, it can distinguish tumor cells from non-tumor cells, especially in cases containing a high number of nonmalignant cells, such as inflammatory tissue, or normal epithelium; 2) It can recognize gain of gene copies owing to polysomies or polyploidies or low-level amplifications. Therefore, to some extent, the differences in amplification frequency could be attributed to the different methodology used. Besides, the difference in the threshold set to distinguish gene amplification, the difference between ESCC cohorts, and tumor heterogeneity might also contribute to the variations of SOX2 gene amplification frequency, which were listed in Paragraph 2, Discussion.

4. Imaging resolution is so poor to judge chromosomal variation in Fig. 1. Please show clearer imaging.

Reply 4: Thank you very much for pointing out this. Figure 1 was revised.

5. Chromosomal variation should be assessed for prognostic relevance separately in stage I and stage II-VI, because it is an independent prognostic factor as well as stage, and it is significantly associated with the stage. This may be the most important finding in this paper.

Reply 5: Thank you very much for your suggestion. Just as in our table 1, the number of patients in Stage I and IV is little. The patients with stage IV were removed in our revised manuscript according to one reviewer's suggestion. The disease free survival analysis were conducted in Stage I, Stage II, and Stage III, respectively (following Table 1-2, Figure 1). The similarly potential survival difference was observed in Stage II and Stage III ESCC, and no complete survival comparison in Stage I. The fewer case number in Stage I had effect on the survival analysis. Chromosomal variation couldn't be assessed for prognostic relevance separately in stage I and stage II-VI, because of the limited case number in Stage I. And this was what we wanted to do in the future.

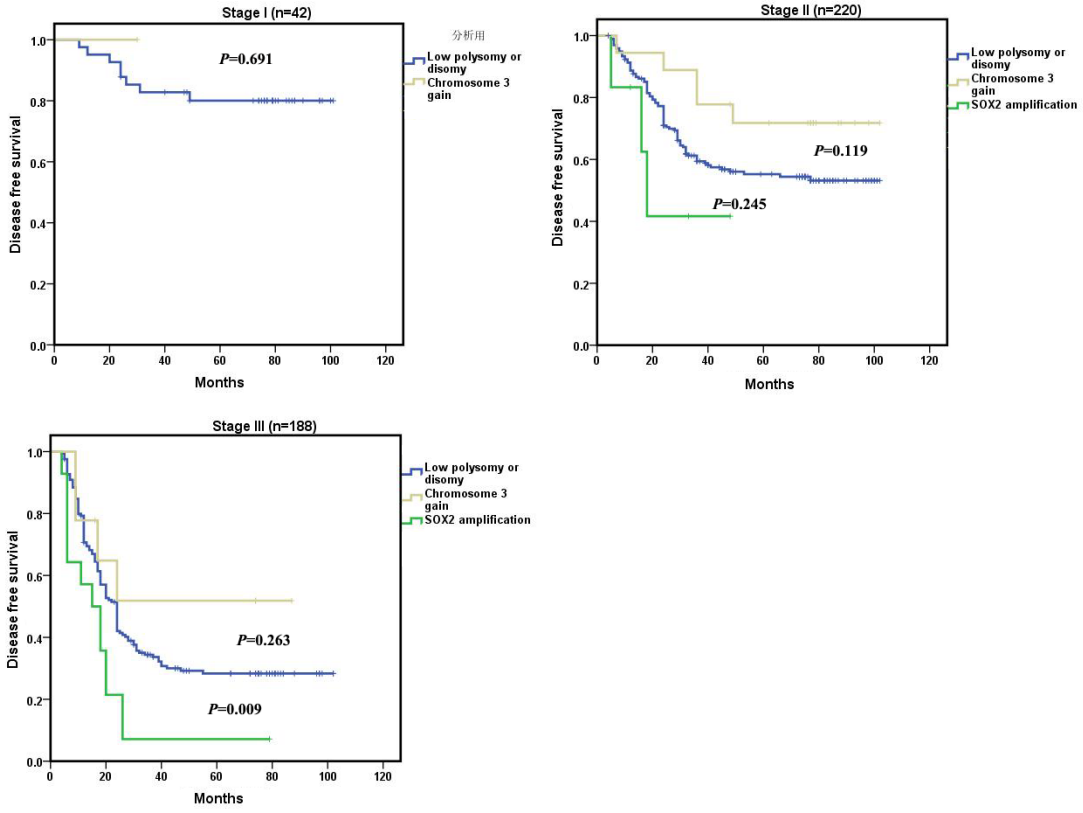
Table 1 Case Processing Summary for DFS

Clinical stage	Chromosomal variation	Total N	N of Events	Censored	
				N	Percent
I	Low polysomy or disomy	41	8	33	80.5%
	Chromosome 3 gain	1	0	1	100.0%
	Overall	42	8	34	81.0%
II	Low polysomy or disomy	196	85	111	56.6%
	Chromosome 3 gain	18	5	13	72.2%
	Sox2 amplification	6	3	3	50.0%

	Overall	220	93	127	57.7%
III	Low polysomy or disomy	165	114	51	30.9%
	Chromosome 3 gain	9	4	5	55.6%
	Sox2 amplification	14	13	1	7.1%
	Overall	188	131	57	30.3%

Table 2 Overall Comparisons for DFS

Clinical stage		Chromosomal variation	Low polysomy or disomy		Chromosome 3 gain		Sox2 amplification	
			Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
I	Log Rank (Mantel-Cox)	Low polysomy or disomy			0.158	0.691		
		Chromosome 3 gain	0.158	0.691				
II	Log Rank (Mantel-Cox)	Low polysomy or disomy			2.433	0.119	1.35	0.245
		Chromosome 3 gain	2.433	0.119			4.631	0.031
		Sox2 amplification	1.35	0.245	4.631	0.031		
III	Log Rank (Mantel-Cox)	Low polysomy or disomy			1.255	0.263	6.79	0.009
		Chromosome 3 gain	1.255	0.263			4.188	0.041
		Sox2 amplification	6.79	0.009	4.188	0.041		



6. English should be proofread in the native speaker.

Reply 6: The revised manuscript was checked carefully by all co-authors. Among our co-authors, Dr Xiaowen Ge ever worked in Laboratory for Translational Research, Harvard Medical School, Cambridge, USA and is fluent in English. The revised words were marked red in the tracking version of the revised manuscripts.

Changes in the text:

Minor

1. Why do you think that SOX2 amplification represents poor prognosis in ESCC, in contrast to lung cancer? Please discuss the differential prognostic significance of the SOX2 gene.

Reply 1: Just as what we listed in Paragraph 3, Discussion, the prognostic significance was contrary in reported different cancers. However, none of the above studies analysis the prognostic difference between chromosome 3 gain (no less than three green signals) and SOX2 amplification (≥4 red target signals in no less than 30% tumor cells and less than three green signals) in their study, which might have an effect on their study to some extent. What's more, as we all know, different molecular might have different influence on the development and progression in different tumors.

2. Letter format should be the same in Table 3 (DFS and OS) as in other portions of this paper.

Reply 2: SOX2 overexpression was revised as SOX2 expression in Table 3. Copy number variation was revised as gene copy number variation in Table 2.

Changes in the text: "SOX2 overexpression" was revised as "SOX2 expression" in Table 3. "Copy number variation" was revised as "gene copy number variation" in Table 2.

Reviewer C

In this manuscript, the authors studied the potential role of SOX2 in ESCC development and progression. 474 ESCC samples were assessed by fluorescence in situ hybridization and immunohistochemistry for SOX2 amplification and protein expression. The authors found that SOX2 amplification is associated with poor progress which is consistent with the findings reported in Liu et al., Cell Stem Cell. 2013 Mar 7;12(3):304-15. This paper needs to be cited. Interestingly, the authors also found that chromosome 3 amplification where the SOX2 gene is located along with other oncogenes like PIK3CA, trp63, etc. The findings are significant and novel, especially chromosome 3 amplification is associated with a good prognosis. It will be informative if the authors can discuss why chrom 3 amplification is associated with a better prognosis given that the amplified genes include multiple oncogenes.

Reply: Thank you very much for your suggestion, the reference (Liu et al., Cell Stem Cell. 2013 Mar 7;12(3):304-15) was added in Line 10, Paragraph 3, Discussion. "We speculated why *Chromosome* 3 gain is associated with a better prognosis might due to *Chromosome* 3 gain include multiple oncogenes." was added in Line 17, Paragraph 3, Discussion.

Changes in the text:

30. Liu K, Jiang M, Lu Y et al. Sox2 cooperates with inflammation-mediated Stat3 activation in the malignant transformation of foregut basal progenitor cells. Cell stem cell 2013, 12(3):304-315.

"We speculated why *Chromosome* 3 gain is associated with a better prognosis might due to *Chromosome* 3 gain include multiple oncogenes." was added in Line 17, Paragraph 3, Discussion.

Reviewer D

Authors investigate the importance of SOX2 amplification, and chromosome 3 gain in esophageal squamous cell carcinoma (ESCC). They found 4.2% ESCCs were found with SOX2 amplification and 12.4% cases with chromosome 3 gain. They demonstrate SOX2 amplification is an independent poorer prognostic factor, but chromosome 3 gain is an independent favorable prognostic factor. The sample size is very large and data analyses are reliable. But, many studies had reported similar results in various cancers, including ESCC. So, the new findings are limited.

There are several issues that should be addressed.

1. Many studies had reported the value of SOX AMPLIFICATION and chromosome 3 gain in ESCC. What are the new meaningful findings in this study?

Reply 1: Thank you very much for your suggestion. With "chromosome 3 gain and esophageal cancer" used in Pubmed, only the following 8 references were found. With the learning of the 8 reference, none of them had an detailed analysis between SOX2 amplification and chromosome 3 gain. Because of our limited knowledge, could you recommend the many related study to us for further study.

1. Gen Y, Yasui K, Zen Y, Zen K, Dohi O, Endo M, Tsuji K, Wakabayashi N, Itoh Y, Naito Y et al: SOX2 identified as a target gene for the amplification at 3q26 that is frequently detected in esophageal squamous cell carcinoma. Cancer genetics and cytogenetics 2010, 202(2):82-93.

2. Hu N, Clifford RJ, Yang HH, Wang C, Goldstein AM, Ding T, Taylor PR, Lee MP: Genome wide analysis of DNA copy number neutral loss of heterozygosity (CNNLOH) and its relation to gene expression in esophageal squamous cell carcinoma. BMC genomics 2010, 11:576.

3. Kang W, Yao HQ, Fang LL, Cai Y, Han YL, Xu X, Zhang Y, Jia XM, Wang MR: [Aneuploid analysis of chromosomes 3, 8, 10, 20 and Y in esophageal squamous cell carcinoma]. Yi chuan = Hereditas 2009, 31(3):255-260.

4. Noguchi T, Kimura Y, Takeno S, Chujo M, Uchida Y, Mueller W, Gabbert HE: Chromosomal imbalance in esophageal squamous cell carcinoma: 3q gain correlates with tumor progression but not prognostic significance. Oncology reports 2003, 10(5):1393-1400.

5. Qin YR, Wang LD, Kwong D, Gao SS, Guan XY, Zhuang ZH, Fan ZM, Deng W, Hu L: [Comparative genomic hybridization: the profile of chromosomal imbalances in esophageal squamous cell carcinoma]. Zhonghua bing li xue za zhi = Chinese journal of pathology 2005, 34(2):80-83.

6. Qin YR, Wang LD, Kwong D, Guan XY, Zhuang ZH, Fan ZM, An JY, Tsao G: [Comparative genomic hybridization of esophageal squamous cell carcinoma and gastric cardia adenocarcinoma in high-incidence region of esophageal carcinoma,

Linzhou Henan]. Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese journal of medical genetics 2004, 21(6):625-628.
7. Yang YL, Chu JY, Luo ML, Wu YP, Zhang Y, Feng YB, Shi ZZ, Xu X, Han YL, Cai Y et al: Amplification of PRKCI, located in 3q26, is associated with lymph node metastasis in esophageal squamous cell carcinoma. Genes, chromosomes & cancer 2008, 47(2):127-136.
8. Yen CC, Chen YJ, Pan CC, Lu KH, Chen PC, Hsia JY, Chen JT, Wu YC, Hsu WH, Wang LS et al: Copy number changes of target genes in chromosome 3q25.3-qter of esophageal squamous cell carcinoma: TP63 is amplified in early carcinogenesis but down-regulated as disease progressed. World journal of gastroenterology 2005, 11(9):1267-1272.

Yes, SOX2 amplification was reported in some studies, with different detection methods. Many studies were based on PCR-related methods. FISH method was used in our study, which enable us 1) visualize individual cells, it can distinguish tumor cells from non-tumor cells, especially in cases containing a high number of nonmalignant cells, such as inflammatory tissue, or normal epithelium; 2) recognize gain of gene copies owing to polysomies or polyploidies or low-level amplifications. Because of the advantage of FISH methods, we found the difference of SOX2 copy number gain and chromosome 3 gain exist in different and same ESCC samples. This finding enable us to explore the prognostic difference between SOX2 amplification and chromosome 3 gain. At present, there was limited study correlating SOX2 amplification and chromosome 3 gain with its clinical significance in large scale of ESCC patients.

2. Authors declaim "This is the first study assessing SOX2 amplification and chromosome 3 gain in .....". But we found this is not the first study.
Reply 2: Thank you for pointing out this. The words were revised in Line 1, Paragraph 5, Discussion.

Changes in the text: "To our knowledge, there is limited study correlating *SOX2* amplification and *chromosome 3* gain with its clinical significance in patients with ESCC."

3. The author excluded patients who had disease progression within three months after surgery. Please provide the reasons.
Reply 3: Thank you for pointing out this. There was some debate about disease progression and coexisting disease within three months. In order to induce the dispute, one patient whether had disease progression or coexisting early esophageal cancer was excluded in our study, according to the suggestion of clinical experts.

4. Please describe the tissue microarray (TMA) construction process in brief.

Reply 4: Thank you for your suggestion. The following sentences were added to describe TMA construction process in Tissue microarray (TMA) construction, Methods.

Changes in the text: "Donor tissues were then manually planted into the recipient block one by one according to the corresponding location indicated by letters and numbers. The planting surface was aggregated on the aggregation instrument. Then the recipient block with the transparent box was placed at 4°C for 10 min until the paraffin could be easily separated from the transparent box. TMA recipient block was taken out and sectioned on a routine microtome machine for further IHC and FISH staining."

5. Authors declaim" this retrospective study consisted of 474 ESCC patients who had undergone esophagectomy without neoadjuvant treatment". But stage IVA in 5.1% of cases. Please provide the reasons. I recommend patients with missing stage information should be excluded.

Reply 5: The 5.1% cases were removed in the revised manuscript.

Changes in the text: All the related data were revised in the revised manuscript.

6. Why there is no ESCC with non-differentiated (Grade four)

Reply 6: A three-tiered system (grade 1, 2,3) is commonly applied in esophageal squamous cell carcinoma. Grade 1 is well-differentiated, Grade 2 is moderately differentiated, and Grade 3 is poorly differentiated.

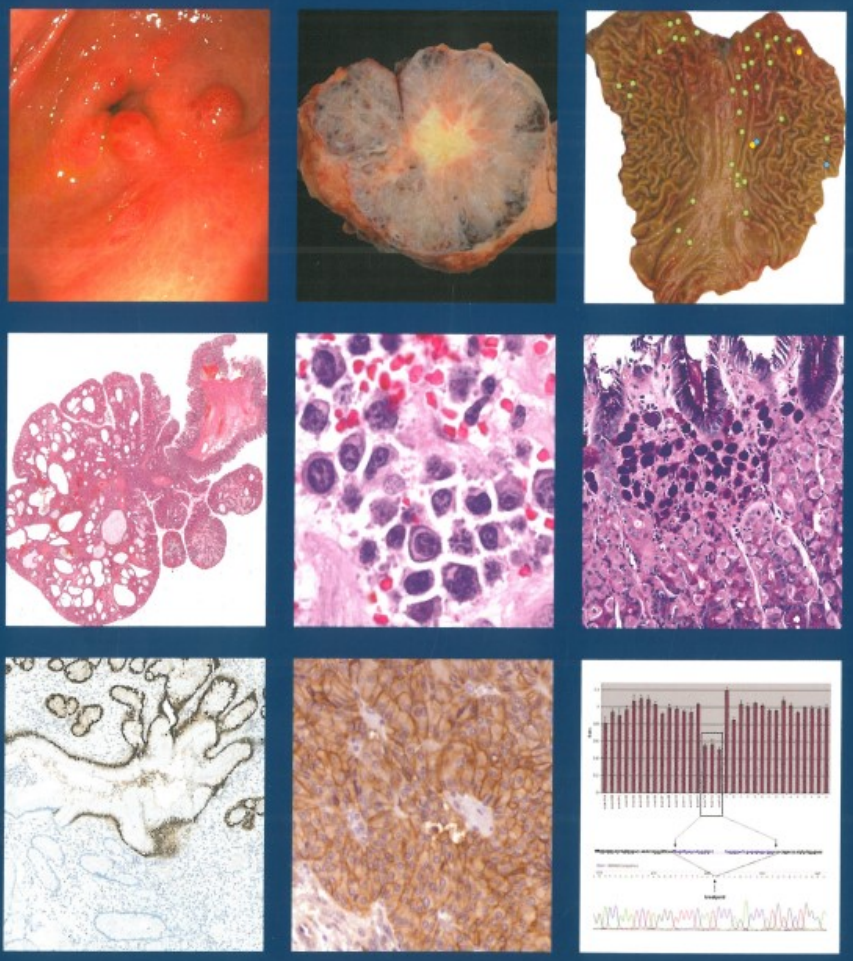
Undifferentiated carcinoma of the esophagus is esophageal epithelial tumor that lacks definite microscopic features of squamous, glandular, or neuroendocrine differentiation. In 2019 WHO, IHC makers are recommended for distinguish undifferntiatied carcinoma from neuroendocrine carcinoma (NEC, CgA, SYN, CD56), poorly differentiated squamous cell carcinoma (p63 and p40), and poorly differentiated adenocarcinoma (mucins). Undifferentiated carcinoma might result from the dedifferentiation of squamous cell carcinoma or adenocarcinoma of esophageal epithelial origin. In the 2019 WHO, undifferentiated carcinoma was a separate chapter, independent of esophageal squamous cell carcinoma. That's to say, it's not appropriate to include undifferentiated carcinoma cases into esophageal squamous cell carcinomas' study.

The following figures about grading of esophageal squamous cell carcinoma and about undifferentiated carcinoma are listed as follows.

WHO Classification of Tumours • 5th Edition

Digestive System Tumours

Edited by the WHO Classification of Tumours Editorial Board



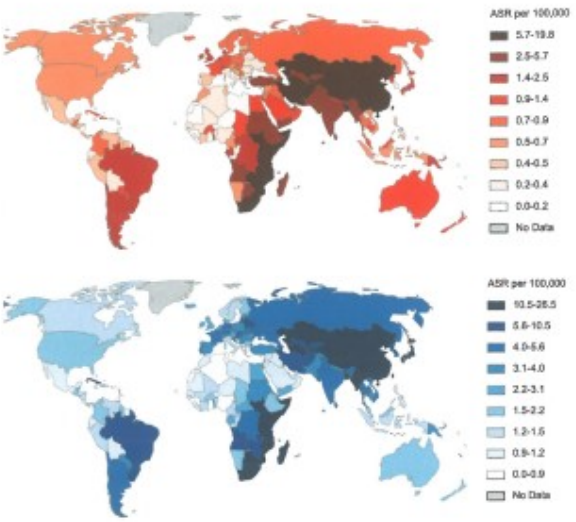


Fig. 2.19 Estimated age-standardized incidence rates (ASRs; World), per 100 000 person-years, of oesophageal squamous cell carcinoma in women (**top**) and men (**bottom**) in 2012.

present in their fifth to eighth decade [1778]. Multiple squamous cell carcinomas can occur in the oesophagus and other parts of the aerodigestive tract (e.g. the oral cavity and oropharynx); this finding is related both to the common exposure of these regions to risk factors such as tobacco smoke and to the rich lymphatic networks in the oesophagus [1775,1779].

Etiology

The etiology of oesophageal squamous cell carcinoma is multifactorial and heavily dependent on the population being studied. The main risk factors are presented in Box 2.03 (p. 48).

Pathogenesis

Oesophageal squamous cell carcinoma develops by stepwise progression, with accumulating genetic abnormalities driving progression from histologically normal squamous mucosa to low-grade intraepithelial neoplasia (dysplasia) to high-grade intraepithelial neoplasia and finally to invasive squamous cell carcinoma. *TP53* mutation is a key early driver mutation [1918]. Genetic changes identified at the intraepithelial neoplasia stage include aneuploidy, copy-number alterations, changes related to the amplification of genes such as *EGFR*, and the silencing of genes such as *CDKN2A* due to promoter hypermethylation [1918]. The specific mutations required for the invasion beyond the basement membrane that is characteristic of invasive squamous cell carcinoma are still unknown. Acquisition of invasive and migratory capability via epithelial–mesenchymal transition is important. *EIF5A2* amplification has been shown to be a factor in inducing this phenotype [1893]. The key genetic abnormalities identified in oesophageal squamous cell carcinoma [2420,8] are summarized in Box 2.04 (p. 49).

Macroscopic appearance

Squamous cell carcinoma often presents at an advanced pathological stage with an ulcerative mass. The most useful macroscopic classification, illustrated in Fig. 2.20, has been provided by the Japan Esophageal Society [1427].

Histopathology

Grading

Squamous cell carcinoma has both vertical and horizontal growth of neoplastic squamous epithelium beyond the basement membrane. Grading is based on the degree of cytological atypia, mitotic activity, and presence of keratinization. A three-tiered system (grades 1, 2, 3) is commonly applied; however, a two-tiered system (grade 1–2 vs grade 3) may be clinically relevant, because the pathological distinction between grade 1 and grade 2 often shows high interobserver variation.

Grade 1 (well-differentiated) squamous cell carcinoma contains enlarged cells with abundant eosinophilic cytoplasm and keratin pearl production. Cytological atypia is minimal and the mitotic rate is low. The invasive margin is pushing and the cells remain well ordered.

Grade 2 (moderately differentiated) squamous cell carcinoma has evident cytological atypia and the cells are less ordered. Mitotic figures are easily identified. There is usually surface parakeratosis, but keratin pearl formation is infrequent.

Grade 3 (poorly differentiated) squamous cell carcinoma consists predominantly of basal-like cells forming nests, which may show central necrosis. The tumour nests consist of sheets or pavement-like arrangements of tumour cells with occasional parakeratotic or keratinizing cells.

Therapy effects

Most patients with advanced oesophageal squamous cell carcinoma are treated with combined preoperative chemotherapy and radiotherapy. This usually induces progressive changes in both the tumour cells and the peritumoural stroma, with macroscopic tumour regression. Cellular changes include nuclear enlargement or shrinkage, nuclear vacuolation, apoptosis, and necrosis. Keratin released from the dying cells may accumulate, undergo dystrophic calcification, and elicit a surrounding giant cell reaction. A neutrophilic or chronic inflammatory cell response may be seen. There is fibrosis and sometimes stromal elastosis. Regional vessels typically show arteriosclerosis.

The extent of tumour regression is an important prognostic factor. It is graded on histological examination by comparing the amount of residual tumour with the amount of therapy-induced fibrosis. The most widely used method of assessing tumour regression grade (TRG) is the Mandard system (see Table 2.02, p. 43) [2036,2708]. Another system relies on the estimated percentage reduction in tumour volume, with < 10% residual tumour constituting a good prognostic finding [244]. Pathological complete response (i.e. complete or nearly complete tumour eradication) is the primary goal of preoperative therapy.

Subtypes

Verrucous squamous cell carcinoma [2461,76,3341] is a subtype often arising in the setting of chronic irritation, oesophagitis, or previous oesophageal injury. Therefore, most cases are identified in the lower third of the oesophagus, as a protuberant mass. Association with HPV51 and HPV11 has been demonstrated in

Oesophageal undifferentiated carcinoma

Kawachi H
Saito T

Definition
Undifferentiated carcinoma of the oesophagus is a malignant oesophageal epithelial tumour that lacks definite microscopic features of squamous, glandular, or neuroendocrine differentiation.

ICD-O coding
8020/3 Carcinoma, undifferentiated, NOS

ICD-11 coding
2B70.Y & XH1YY4 Other specified malignant neoplasms of oesophagus & Carcinoma, undifferentiated, NOS

Related terminology
None

Subtype(s)
Lymphoepithelioma-like carcinoma (8082/3)

Localization
This carcinoma is most often located in the lower oesophagus or the oesophagogastric junction {3063}.

Clinical features
The most common symptom is progressive dysphagia, followed by gastro-oesophageal reflux, weight loss, and anaemia {3063}.

Epidemiology
The reported relative prevalence varies widely, from 0.18% to 4% of oesophageal carcinomas {2709,3427}; this apparent variation is probably related to the lack of diagnostic criteria. In reported US series, patients ranged in age from 39 to 84 years (mean: 65.5 years) and were predominantly male {3063}.

Etiology
Unknown

Pathogenesis
Undifferentiated carcinoma most likely results from the dedifferentiation of squamous cell carcinoma or adenocarcinoma of oesophageal epithelial origin. In the largest study of undifferentiated carcinomas to date, 12 of 16 cases (75%) were associated with Barrett oesophagus, and some of those carcinomas contained focal glandular differentiation {3063}.

Macroscopic appearance
The tumours are exophytic, with raised edges, and they are centred in either the oesophagus or the oesophagogastric junction {3063}. In most cases, there are central areas of depression and ulceration.

Histopathology
The tumour cells form variably sized nests and sheet-like arrangements. The cells are medium-sized to large, with poorly defined amphophilic to slightly eosinophilic cytoplasm imparting a syncytial-like appearance. Cytologically, the nuclei are oval and vesicular. Large pleomorphic nuclei and multinucleated giant cells mimicking osteoclasts or rhabdoid cells are occasionally found {3063}.

Lymphoepithelioma-like carcinoma {3273} is considered to be a distinct subtype of undifferentiated carcinoma. Most reported cases have been from Japan and clinically resemble conventional squamous cell carcinoma {611,2305}. This subtype is characterized by a sheet-like arrangement of large epithelioid cells with prominent nucleoli and indistinct cell borders. The tumour is surrounded by a characteristic inflammatory infiltrate that is rich in lymphocytes and plasma cells. Unlike

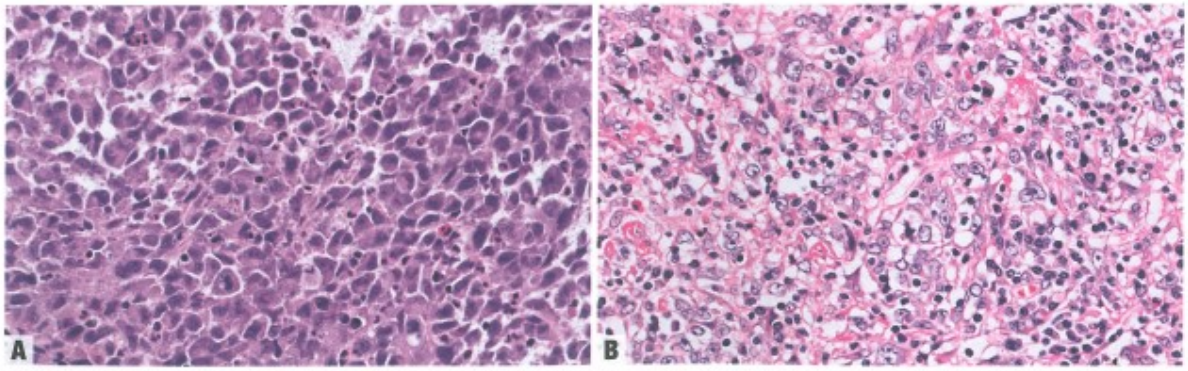


Fig. 2.26 Oesophageal undifferentiated carcinoma. **A** The nuclei of the tumour cells are pleomorphic; rhabdoid cells are occasionally found. **B** The lymphoepithelioma-like carcinoma subtype is characterized by sheet-like arrangement of large epithelioid cells with prominent nucleoli and indistinct cell borders; a characteristic inflammatory infiltrate rich in lymphocytes and plasma cells surrounds the tumour.

7. Some clinical and pathological information is missing, such as postoperative treatments. Please add those significant clinicopathological features. The authors simplify the survival analyses. Please describe the multivariable analyses clearly,

forward or stepwise or entry? What are the standards of varibles enrolled into survival COX model in this study?

Reply 7: Thank you for your suggestion. In Line 9, Statistical Analysis, Methods, we have mentioned "Factors with a *P* value <0.05 for either the OS or DFS, which met the proportional hazards criteria in the univariate analysis, were included in a stepwise multivariate Cox regression." According to your suggestion, the sentence "With variables that were found to be significant in the univariate analyses ($P<0.005$), the multivariate analysis using the cox proportional hazard model was performed." was added in Line 13, Paragraph 2, Survival analyses, Results.

Changes in the text: "With variables that were found to be significant in the univariate analyses ($P<0.005$), the multivariate analysis using the cox proportional hazard model was performed." was added in Line 13, Paragraph 2, Survival analyses, Results.

8. Previous studies had analyzed the prognostic value of SOX2 amplification and chromosome 3 gain in ESCC. We recommend authors perform cellular research to explore biological functions and experimental validation.

Reply 8: Thank you for pointing out this. That's what we want to do in the future.