Peer Review File

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**Reviewer A**

The paper titled "Esophageal carcinoma with SMARCA4 mutation: A narrative review for this rare entity" is interesting. Esophageal carcinoma with SMARCA4 mutation is an overly aggressive disease, and further research on the affected molecular pathway may help improve its prognosis. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: What significant challenges may SMARCA4 mutated esophageal cancer pose to surgical pathologists in diagnosis due to its diverse morphology and immune characteristics? Suggest adding relevant content.

Reply: We agree with the reviewer's opinion, and this is one of our motivations to write this manuscript. We revised the first paragraph of the Considerations for differential diagnosis section (page 15, line 11), and it reads:

"Owing to its rare occurrence, the pathological diagnosis of esophageal carcinoma with *SMARCA4* mutations is extremely challenging, especially in small tissue fragments from biopsies, due to the following reasons. First, esophageal carcinoma with *SMARCA4* mutations can show different histological patterns, including epithelioid, rhabdoid, and glandular features. Second, its immunohistochemical patterns are highly variable, especially for those widely utilized cytokeratins such as AE1/3, Cam 5.2, CK-cocktail, CK-Oscar. Third, since it is a relatively newly established entity, SMARCA4/BRG1 immunohistochemistry is not widely available, and surgical pathologists had limited experiences of interpreting such stains."

Comment 2: There have been many studies on esophageal carcinoma. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply: We agree with the reviewer's opinion. We revised the third paragraph of the Introduction section (page 5, line 15), it reads:

"However, only a few case series and case reports of esophageal carcinoma with *SMARCA4* mutations have been published in the English literature (23, 25-32). To the best of our knowledge, there are no previously published comprehensive review articles for esophageal carcinoma with *SMARCA4* mutations covering its epidemiological, clinical, pathological, and molecular features. The rarity of the disease poses significant diagnostic challenges for surgical pathologists and can potentially lead to delayed or suboptimal patient care. Herein, we reviewed the available literature on esophageal carcinoma with *SMARCA4* mutations to discuss its epidemiological, clinical, pathological, and molecular features with diagnostic challenges, as well as provide an overview of its treatment and prognosis."

Comment 3: There are many databases. Why did the author only select PubMed, Scopus, and Google Scholar databases in this study for searching? Please explain the reason.

Reply: PubMed, Scopus, and Google Scholar are the most commonly used ones, and more importantly, they are free of charge for everyone from anywhere in the world. However, we agree with the reviewer that there are many other databases. To increase the scope of our search, we additionally searched Ovid database, which returned the same searching results. We revised the Method section (page 6, line 14) and Table 1, and it reads:

"A search was conducted in the PubMed, Scopus, Ovid, and Google Scholar databases using selected keywords (Table 1). The search was originally conducted in August 2023 and updated in Jan 2024."

Comment 4-1: What is the chromosome stability of SMARCA4 mutation patients?

Reply: We agree with the reviewer's question regarding chromosome stability in patients with esophageal carcinoma with *SMARCA4* mutations. To address this comment, we added a whole new second paragraph in the Introduction section (page 4, line 13), and it reads:

"Numerous studies have shown that *SMARCA4* mutations mainly contribute to carcinogenesis in two aspects. First, SMARCA4/BRG1 proteins were direct regulators for transcriptional activation of genes related to carcinogenesis. For example, loss of SMARCA4/BRG1 has been shown to cause overexpression of oncoprotein MYC in lung cancer, which enabled cancer cells to sustain undifferentiated gene expression programs and prevented its response to environmental stimuli (13). In breast cancer cells, loss of SMARCA4/BRG1 reduced its binding to BRCA1 protein, which disrupted the tumor suppresser function of BRCA1 (14). Deletion of SMARCA4/BRG1 also caused increased expression of CD44 in cell cultures and decreased CDK4/6 kinase activity in ovarian cancer cells, resulting in cell cycle progressing, increased tumor growth, and metastasis (15-16). Secondly, SMARCA4/BRG1 protein played important roles in DNA processing as an essential component of chromatin remodeling complex in addition to its transcription regulating roles, which included regulating and promoting DNA repair by repositioning nucleosomes and recruiting other DNA repair proteins (17–19). In human lung cancer and breast cancer, the absence of SMARCA4/BRG1 protein has been demonstrated to be a major cause of genome instability (20-22)."

Comment 4-2: What is the correlation with the activated immune phenotype? It is suggested to add relevant content.

Reply: We agree with the reviewer's question regarding activated immune phenotype in patients with esophageal carcinoma with *SMARCA4* mutations. To address this comment, we revised the Molecular features and pathogenesis section and added a new third paragraph (page 14, line 21), and it reads:

"In lung, thoracic SMARCA4-deficient undifferentiated tumors were shown to be mainly immune desert tumors with no tertiary lymphoid structures, and with limited efficacy of immune checkpoint inhibitors (36). The results of immune phenotypes were limited in esophageal carcinoma with *SMARCA4* mutations but showed similar trends. The tumor mutation burden was low in all four patients reported by Cui et al., while PD-L1 immunohistochemistry was performed in three patients, and one patient (33%) showed positive PD-L1 expression (combined positive score = 10) (25). Mismatch repair protein immunohistochemistry was performed in seventeen patients from four

studies, and all showed intact expression of MLH1, PMS2, MSH2, and MSH6 proteins (25, 28, 29, 31)."

Comment 5: What is the full spectrum and frequency of SMARCA4 mutations in esophageal carcinoma? Suggest adding relevant content.

Reply: We agree with the reviewer's question regarding the full spectrum and frequency *SMARCA4* mutations. To address this comment, we revised the first paragraph of Molecular features and pathogenesis section (page 12, line 8), and it reads:

"Schallenberg et al. reported that nineteen of 563 (3.4 %) esophageal adenocarcinoma patients showed SMRACA4 protein loss on immunohistochemistry (32). However, as a newly established and rare disease entity, the exact incidence of esophageal carcinoma and *SMARCA4* mutations remains unknown. In lung cancer, the biallelic inactivation of *SMARCA4* drives tumorigenesis, mainly through nonsense, frameshift, missense, and splice-site mutations; deletions; and loss of heterozygosity with a second mutation (8, 33). Similarly, Neil et al. provided a detailed description of the mutation profiles of esophageal and gastric carcinomas with *SMARCA4* mutations (27). Pathogenic mutations were divided into two groups. Group 1 includes nonsense, frameshift, and splice site mutations, which are predicted to lead to premature protein truncation, whereas group 2 consists of those that had been previously identified as pathogenic *SMARCA4* mutational hotspots in large pan-cancer cohorts, including codons G782, G784, K785, T786, A791, P811, L815, E821, Y860, E861, E882, H884, R885, T910, P913, E920, R966, R973, R979, F1102, R1135, R1157, G1159, G1160, G1162, D1177, A1186, R1189, R1192, G1194, G1232, D1235, and R1243 (4, 34, 35). Unfortunately, the differences in the mutation profiles of esophageal and gastric carcinomas were not distinguished. Among the four patients evaluated by Cui et al., three harbored *SMARCA4* sub-gene deep deletions, whereas one had an R1192C missense mutation. In the study by Gupta et al., the sequencing data of three patients were available, including one with copy-neutral loss of heterozygosity of *SMARCA4*, one with loss of chromosome 19 that harbored *SMARCA4* mutations, and one with *SMARCA4* subgene focal deletion."

Comment 6: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Thoracic SMARCA4-deficient undifferentiated tumor-a case of an aggressive neoplasm-case report, PMID: 35118344". It is recommended to quote this article.

Reply: We agree with the review's request and added this case report into the manuscript as the new Refence 6. The reason we did not include this case report in the analysis is that we only analyzed the cases from esophagus, and this case report is from lung.

Comment 7: In the introduction of the manuscript, the author needs to increase the core role of SMARCA4 in tumor genesis and development.

Reply: We agree with the reviewer's request regarding the core role of SMARCA4 in tumor genesis and development. To address this comment, we added a whole new paragraph in the Introduction section (page 4, line 13), and it reads:

"Numerous studies have shown that *SMARCA4* mutations mainly contribute to carcinogenesis in two aspects. First, SMARCA4/BRG1 proteins were direct regulators for transcriptional activation of genes related to carcinogenesis. For example, loss of SMARCA4/BRG1 has been shown to cause overexpression of oncoprotein MYC in lung cancer, which enabled cancer cells to sustain undifferentiated gene expression programs and prevented its response to environmental stimuli (13). In breast cancer cells, loss of SMARCA4/BRG1 reduced its binding to BRCA1 protein, which disrupted the tumor suppresser function of BRCA1 (14). Deletion of SMARCA4/BRG1 also caused increased expression of CD44 in cell cultures and decreased CDK4/6 kinase activity in ovarian cancer cells, resulting in cell cycle progressing, increased tumor growth, and metastasis (15-16). Secondly, SMARCA4/BRG1 protein played important roles in DNA processing as an essential component of chromatin remodeling complex in addition to its transcription regulating roles, which included regulating and promoting DNA repair by repositioning nucleosomes and recruiting other DNA repair proteins (17–19). In human lung cancer and breast cancer, the absence of SMARCA4/BRG1 protein has been demonstrated to be a major cause of genome instability (20-22)."

**Reviewer B**

Comment 1: In the text, references should be cited consecutively.

Reply: Thanks for pointing out. We changed the reference number from “24” to “13” at page 4, line 19.

Comment 2. If references are cited in tables or figure legends, number them according to the first identification of the table or figure in the text.

Reply: Thanks for pointing out. We made sure that all references are numbered continuously in the main text. All the references mentioned in our tables have already been mentioned in the main text and therefore have already been numbered.

Comment 3. “Indeed, in the first case report of esophageal carcinoma with SMARCA4 mutation conducted by **Kilic** et al., the initial impression was hematolymphoid malignancy owing to the weak Pax5 immunoreactivity (**28**).” The author name should be checked for accuracy and align with the reference cited.

Reply: Thanks for pointing out. We changed the reference number from “28” to “23” at page 14, line 12.

Comment 4. Table 1: Regarding “Date of search”, the date when the action of searching was made.

Reply: Thanks for pointing out. We revised Table1: “Date of search” and it reads “ Inception to Jan 25, 2024”

Comment 5. Table 3: A table should not have multiple sets of column headers.

Reply: Thanks for pointing out. Due to the length of Table 3, we revised it into one set of column headers and continued at the next page as “Table 3 continued”. Please let us know if it meets the editorial requirements, we can change it again according to editor’s opinions.

Comment 6. Please provide the definition of + in the explanatory legend of Table 2 and +/MMR/ Her2/ERBB2/PD-L1/CPS in Table 3.

Note: if there are any genes used in the entire article, they should be italicized.

Reply: Thanks for point out. Explanatory legend for all Tables are revised to include the following information, “+, positive; SMARCB1, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1; MMR, mismatch repair proteins; ERBB2, erb-b2 receptor tyrosine kinase 2 protein; PD-L1, programmed cell death 1 ligand 1 protein; CPS, combined

positive score“. Additionally, we confirmed the all genes’ names are italicized in the article, while proteins’ name are not italicized.