Proceedings of the 4th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/40/20241237

The impact of mutations in key genes on the pathogenesis of esophageal cancer

Xueji Shi

West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Sichuan, China, 610207

Sxj3059973018@tom.com

Abstract. Esophageal cancer (EC) ranks seventh among all cancers in terms of frequency and is the sixth leading cause of cancer-related deaths worldwide. The 5-year relative survival rate for esophageal cancer stands at a dismal 21%, which is nearly the lowest among all cancers. In China alone, over 477,900 individuals are diagnosed with EC annually. Advances in whole-genome sequencing technologies have enabled researchers to identify numerous gene mutations across the entire gene sequence in EC patients. This paper aims to consolidate current knowledge on several key genes implicated in EC, as revealed by whole-genome sequencing of patient biopsies. By examining research papers published within the last decade, this study captures the main findings and elucidates the influence of mutations in SOX2, TP53, NOTCH1, SMAD4, and CDKN2A on the formation, progression, and prognosis of both esophageal adenocarcinomas and squamous cell carcinomas. The insights gained are expected to contribute positively to the overall comprehension of esophageal cancer.

Keywords: esophagus cancer, SOX2, TP53, NOTCH1, SMAD4, CDKN2A

1. Introduction

Esophageal cancer (EC) is categorized into two primary histological types: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). Esophageal adenocarcinoma (EAC) develops in the glandular cells that line the lower part of the esophagus, usually close to the stomach, and is more common in Western nations. Risk factors commonly associated with it include chronic acid reflux, gastroesophageal reflux disease (GERD), Barrett's esophagus, and persistent heartburn. ESCC originates from the squamous epithelium of the upper esophagus and is linked to heavy alcohol intake, smoking, and organ donation. ESCC is the most common subtype in China, showing significant geographical clustering in areas such as the Taihang Mountains, Xinjiang province, and the Chaoshan district.

A multitude of research endeavors have concentrated on elucidating the role of specific genes in the etiology, progression, and prognosis of EC. These studies involve whole-genome sequencing of esophageal cancer biopsies from hospitalized patients, juxtaposed with sequencing of adjacent healthy tissue, to identify differentially expressed genes. Certain genes, including SOX2, TP53, NOTCH1, SMAD4, and CDKN2A, have emerged as recurrent themes across multiple investigations. The paper examines the latest findings on five genes commonly associated with esophageal cancer. It aims to synthesize their roles in the pathogenesis, clinical course, and prognostic outcomes of both

DOI: 10.54254/2753-8818/40/20241237

adenocarcinoma and squamous cell carcinoma of the esophagus. In essence, a comprehensive understanding of the impact of these frequently mutated genes is pivotal for enhancing patient care, advancing our scientific grasp of EC, and ultimately, mitigating the global impact of this formidable disease.

2. Analysis of five genes

2.1. SOX2

The SOX2 gene is highly conserved across many species, with the human SOX2 gene located on chromosome 3q and encoding a protein of 317 amino acids [1]. The Sry-related HMG box (SOX) proteins constitute a substantial family, with SOX2 being a member of the SOXB1 subgroup and a transcription factor characterized by an SRY-containing homeobox [2].

SOX2, a protein produced by one of the Yamanaka factors that induce pluripotent stem cells, is crucial for maintaining the stemness of embryonic stem cells and adult pluripotent and multipotent stem cells. Additionally, disruptions in SOX2 are frequently associated with various types of cancer, including esophageal cancer, breast cancer, and liver cancer, among others [1].

In the context of esophageal cancer (EC), Sox2 is widely recognized as a key player in tumorigenesis. A recent study observed that increased expression of SOX2, facilitated by KLF5, can lead to a reliance on the RNA editing enzyme ADAR1 by altering the epigenome of esophageal squamous cell carcinoma (ESCC). This process also results in the upregulation of the IL6 receptor pathway and activation of STAT3 [2].

Chlorogenic acid, a chemical found in plants and derived from traditional Chinese medicine (TCM), has demonstrated the ability to hinder the progression of esophageal squamous cell carcinoma in laboratory and animal studies. It inhibits the movement and penetration of ESCC cells in a lab setting by decreasing the levels of SOX2 and BMI1, indicating its possible use as a predictive indicator for ESCC therapy [3]. The Regulator of chromosome condensation 2 (RCC2), a centrosome-located protein, is overexpressed in esophageal cancer and is associated with tumorigenicity. RCC2 upregulates the activity and expression of SOX2, thereby promoting esophageal tumor growth [4].

Recent research indicates that SOX2OT, a long non-coding RNA, plays a role in preserving stem cell characteristics and promoting cancer development in different types of carcinomas, such as ESCC. Suppressing SOX2OT lncRNA has been found to inhibit esophageal tumorspheres [5]. SOX2 + cells have been implicated in causing hyperplasia of the esophagus and stomach by activating the CXCR2 pathway. In this study, SOX2+ cells are considered as potential candidates for the origin of esophageal cancer [6]. Incidentally, SOX2 + cells have also been shown to induce the formation of propagating medulloblastomas [6].

These research and data clearly demonstrate that SOX2 is crucial in the development of esophageal tumors. Current understanding of the importance of SOX2 and its involvement in cancer advancement is insufficient, and treatment methods focusing on transcription factors such as SOX2 are restricted.

2.2. TP53

TP53 mutations are common and found in around 50% of malignancies. This transcription factor is crucial in controlling the expression of more than 500 genes, which are essential for processes such as cell cycle regulation, DNA damage repair, and apoptosis, all of which are important in tumor formation [7].

Under normal, unstressed conditions, TP53 expression is minimal, caught in a negative feedback loop. However, when cellular stress occurs, such as during DNA damage repair, TP53 expression is significantly upregulated through various cellular signaling pathways [7]. The majority of TP53 mutations in tumor development result in a complete loss of protein function, typically due to a single amino acid substitution within the DNA binding domain [7]. Extensive research has implicated TP53 in the tumorigenesis of esophageal cancer (EC). A study has indicated that TP53 can serve as a prognostic indicator for disease progression in patients with Barrett's esophagus, a precursor to esophageal

DOI: 10.54254/2753-8818/40/20241237

adenocarcinoma, regardless of dysplasia diagnosis. TP53 mutations are consistently associated with abnormal p53 immunohistochemical (IHC) expression, which is emerging as a promising biomarker for the progression of Barrett's esophagus [8]. In squamous esophageal cell carcinomas, biallelic TP53 disruption is a probable event and may represent the cell-of-origin for squamous cell carcinoma, as suggested by multiregional sequencing [9].

During the process of carcinogenesis, p53 mutations do not initiate tumor formation but rather promote subsequent stages of tumorigenesis, influenced by clonal competition [9]. Patients with biallelic TP53 mutations exhibit shorter non-progression survival period than those with TP53 pathway wild-type tumors. Furthermore, alterations in the TP53 pathway are statistically associated with the response to neoadjuvant therapy [10].

Severe malfunction of TP53 is associated with negative results. Targeting p53 or its regulators is a viable therapeutic approach to halt the advancement of Barrett's esophagus. A study found that the absence of RNF128 Iso2–UBCH5C and the presence of the Iso1–UBCH5A complex can increase the stability of mutant p53, potentially boosting the survival chances of esophageal cancer patients [6]. A new study has shown that high expression of p53 protein is predictive of poor survival in esophageal squamous cell carcinoma patients, despite ongoing arguments about the prognostic utility of p53 and TP53 as markers for this kind of cancer and their connection to clinical outcomes. This study employed various approaches such as whole-genome sequencing (WGS), whole-exon sequencing (WES), regionally targeted sequencing (TRS), and IHC to investigate the correlation between TP53 mutation or p53 protein expression with the prognosis of ESCC [11].

The critical role of TP53 in esophageal cancer formation is widely acknowledged, and the current understanding of this transcription factor offers a compelling avenue for creating specific therapies to improve the poor prognosis associated with this disease.

2.3. NOTCH1

The Notch family consists of four highly conserved single-pass transmembrane receptors that are crucial for a range of cellular functions such as embryonic development, cell proliferation, apoptosis, and differentiation [12]. The Notch signaling pathway, which is preserved in various species, controls communication between neighboring cells using receptors located on the cell membrane [12].

Notch1, derived from the NOTCH1 gene located at chromosomal position 9q34.3, exhibits the typical structure of type 1 transmembrane proteins, featuring an extracellular domain, many epidermal growth factor-like (EGF) repeats, and an intracellular domain. Notch1 plays a crucial part in normal physiological functions and is also a critical factor in the development of several types of cancer, including prostate cancer, T-cell acute lymphoblastic leukemia, breast cancer, and esophageal cancer [12].

NOTCH1 mutation is one of the most common mutations seen in esophageal cancer (EC) [13]. Unlike other gene variants that support tumor growth, recent research indicates that NOTCH1 mutations can hinder carcinogenesis and stimulate clonal expansion in normal esophageal epithelium. These results indicate that inhibiting NOTCH1 could be a promising therapeutic strategy for avoiding esophageal squamous cell cancer [14].

A recent study has confirmed the significance of NOTCH1 in healthy esophageal tissue. It suggests that the lack of NOTCH1 and the existence of CDKN2A deletion can forecast the advancement of esophageal abnormalities. NOTCH1 mutations and the presence of CDKN2A can serve as biomarkers for identifying persons at high risk for esophageal cancer [15]. Small cell carcinoma of the esophagus (SCCE) is identified by frequent NOTCH1 mutations, which lead to an immunosuppressive environment within the tumor and show potential for the advancement of immune-based cancer treatments. The activation and maintenance of CD8+ T lymphocytes are influenced by the NOTCH pathway, with NOTCH1 being involved [16].

NOTCH1 exhibits significant differences between physiologically normal mucosa (PNM) and tumors of esophageal squamous cell carcinoma (ESCC) [17]. The Notch signaling pathway has been found to enhance the upregulation of KLF4, facilitating the transformation and differentiation of

DOI: 10.54254/2753-8818/40/20241237

esophageal cells towards Barrett's esophagus-like metaplasia. This represents a novel mechanism by which the Notch signaling pathway contributes to the evolution of Barrett's esophagus [18].

In summary, NOTCH1 functions as a tumor suppressor, suggesting that NOTCH1 inhibitors may have the potential to slow down tumor growth. Further research is warranted to elucidate the precise signaling pathways involved and their interactions with other pathways, as well as to determine whether new drugs targeting NOTCH1 could protect against esophageal cancer (EC).

2.4. SMAD4

SMAD4, sometimes referred to as SMAD family member 4, mothers against decapentaplegic homolog 4 (DPC4), or Deleted in Pancreatic Cancer-4 (DPC4), is a widely preserved protein present in all metazoans. The SMAD4 gene is located on chromosome 18q21.1 and consists of 54,829 base pairs in humans. SMAD4, belonging to the second class of the SMAD family, is a 552-amino acid polypeptide with a molecular weight of 60 kilodaltons (kDa). The protein has two functional domains, MH1 and MH2, and is essential for regulating multiple cellular processes such as differentiation, embryonic development, apoptosis, and the cell cycle via influencing the transforming growth factor-beta (TGF−β) pathway. Several research in the field of esophageal squamous cell carcinoma (ESCC) have utilized protein expression tests on patient samples to investigate the involvement of SMAD4 in this specific malignancy.

An immunohistochemical investigation examined the expression of Smad4 and TGF−β1 proteins in 258 patients with esophageal squamous cell carcinoma. SMAD4 expression may predict tumor development, including tumor depth and lymph node metastasis, although it is not an independent prognostic marker [19]. Reduced Smad4 expression in the TGF−β signaling pathway has been noted when esophageal squamous cell cancer advances. An immunohistochemical examination of Smad4 in surgical samples from 80 patients demonstrated a notable inverse relationship between Smad4 levels and the extent of tumor invasion and disease stage [20].

A recent study has proposed that the combined action of Smad4 and PTEN may prevent the development of antral squamous cell carcinoma by synergistically inducing cell cycle inhibitors. This conclusion is drawn from studies in mice with double knockouts for Smad4 and PTEN, which exhibited increased esophageal and antral epithelial proliferation and rapid antral tumor formation [21]. The latest research has provided insight into the upstream regulation of SMAD4. The transcription factor c-Jun controls the expression of Smad4, leading to the invasive metastasis of esophageal squamous cell carcinoma by influencing the expression of pre-miR-183. Targeted inhibition of miR-183 could be a feasible therapeutic technique due to its substantial role in tumor growth and invasion by targeting Smad4 [22].

In summary, while there is a consensus on the involvement of SMAD4 in esophageal cancer, more detailed and comprehensive studies are necessary to fully understand its role in the development, progression, and metastasis of esophageal cancer.

2.5. CDKN2A

Cyclin-dependent kinase inhibitor 2A (CDKN2A), also known as CDKN2A, is expressed in a variety of tissues and cell types and encodes two proteins: the INK4 family member p16 and p14arf. Both p16 and p14arf function as tumor suppressors by regulating the cell cycle. P16 inhibits the activity of CDK4 and CDK6 by binding to them, thereby preventing the cell from continuing the cycle and dividing. Additionally, p14arf helps protect the well-known tumor suppressor p53 from degradation. Situated on chromosome 9p21, CDKN2A is frequently implicated in tumorigenesis, as somatic mutations in CDKN2A are prevalent in many human cancers [23].

In a study examining genetic alterations in CDKN2A and CDKN2B among 56 esophageal squamous cell carcinoma (ESCC) cases from a high-risk Chinese population, 32% of patients (18 of 56) exhibited intragenic allelic loss at the CDKN2A polymorphic locus. These findings suggest that CDKN2A mutations and allelic loss may contribute to the development of ESCC [23]. Another research project examined the expression of CDKN2A/RB1 in tumor and lung tissue samples from healthy individuals,

DOI: 10.54254/2753-8818/40/20241237

those exposed to risk factors (such as alcoholism and smoking), and patients with ESCC. The researchers noted that the level of p16 expression rose in correlation with the severity of histological abnormalities in biopsies from persons exposed to risk factors or those with cancer. As a result, it was concluded that the esophagus lining might be more prone to cancer when exposed to risk factors that trigger the production of these proteins [24].

A third study investigated the correlation between two genetic variations in the 9p21 locus, CDKN2A/B (rs10811661 and rs1333049), and its connection to ESCC in 273 people. The researchers found that the TT genotype of rs10811661 was more common in patients with ESCC compared to the control group and was associated with tumor size. The CC genotype of rs1333049 was associated with decreased overall survival in patients with ESCC. The research found that genetic variations at the CDKN2A/2B locus are linked to a poor outcome in individuals with esophageal squamous cell carcinoma [25].

Moreover, mutations in the CDKN2A gene were discovered to impact the survival duration of patients with ESCC. The p53 gene was the most commonly altered gene in the group, with CDKN2A following closely behind. Poor postoperative prognosis in patients with primary advanced esophageal squamous cell carcinoma may be coincidentally linked to CDKN2A deletion [26].

In summary, CDKN2A exerts a detrimental effect on patients with ESCC, influencing both disease progression and prognosis. Therefore, ongoing research into CDKN2A is warranted, as it may emerge as a potential therapeutic target for ESCC.

3. Conclusion

Esophageal squamous cell carcinoma (ESCC) is the leading form of esophageal cancer in Western countries, while esophageal adenocarcinoma (EAC) predominates in other regions. This review consolidates findings from articles that have utilized whole-genome sequencing of esophageal cancer samples. It identifies the five most frequently implicated genes across studies and provides a summary of their influence on the incidence, progression, and prognosis of esophageal cancer, along with insights into some of the underlying molecular mechanisms.

This paper recognizes two main limitations. The literature evaluated may not cover the total range of each gene's involvement and its matching protein's function in esophageal cancer due to potential omissions of important papers, resulting in incomplete information. The review concentrates on only five genes, despite esophageal cancer being linked to numerous genetic variables. Assuming that a single gene mutation arises independently during a patient's lifetime is not feasible, and other contributing factors cannot be completely excluded. Further study is necessary to confirm the practical consequences of the conclusions derived from this assessment.

It is rarely practical in clinical practice to guarantee that a single gene mutation is the only genetic occurrence in a patient's lifetime, and the influence of other risk factors is sometimes unavoidable. Therefore, further research is needed to validate the practical significance of the review's findings. Esophageal cancer is common in some regions of China, presenting a difficult disease with a poor prognosis and a low five-year survival rate. Esophageal cancer lacks effective targeted treatments, and standard therapy can lead to discomfort and reduce patients' quality of life. It is crucial to continue researching esophageal cancer through genomic studies and creative therapy options by advancing research approaches.

References

[1] Novak D, Hüser L, Elton J J, et al. SOX2 in development and cancer biology[C]. Seminars in cancer biology, 2020: 74-82.

[2] Wu Z, Zhou J, Zhang X, et al. Reprogramming of the esophageal squamous carcinoma epigenome by SOX2 promotes ADAR1 dependence[J]. Nature Genetics, 2021, 53(6): 881-894.

[3] Zhan Y, Li R, Feng C, et al. Chlorogenic acid inhibits esophageal squamous cell carcinoma growth in vitro and in vivo by downregulating the expression of BMI1 and SOX2[J]. Biomedicine & Pharmacotherapy, 2020, 121.

[4] Calderon-Aparicio A, Yamamoto H, De Vitto H, et al. RCC2 Promotes Esophageal Cancer Growth by Regulating Activity and Expression of the Sox2 Transcription Factor[J]. Mol Cancer Res, 2020, 18(11): 1660-1674.
[5] Haghi B, Saghaeian Jazi M, Khosravi A, et al. SOX2OT lncRNA Inhibition Suppresses the Stemness Characteristics of Esophageal Tumorspheres[J]. Non-coding RNA, 2022, 8(6): 80.
[6] Hishida T, Vazquez-Ferrer E, Hishida-Nozaki Y, et al. Mutations in foregut SOX2+ cells induce efficient proliferation via CXCR2 pathway[J]. Protein & cell, 2019, 10(7): 485-495.
[7] Wang Z, Strasser A, Kelly G L. Should mutant TP53 be targeted for cancer therapy?[J]. Cell Death & Differentiation, 2022, 29(5): 911-920.
[8] Redston M, Noffsinger A, Kim A, et al. Abnormal TP53 predicts risk of progression in patients with Barrett's esophagus regardless of a diagnosis of dysplasia[J]. Gastroenterology, 2022, 162(2): 468-481.
[9] Murai K, Dentro S, Ong S H, et al. p53 mutation in normal esophagus promotes multiple stages of carcinogenesis but is constrained by clonal competition[J]. Nature Communications, 2022, 13(1): 6206.
[10] Sihag S, Nussenzweig S C, Walch H S, et al. The Role of the TP53 Pathway in Predicting Response to Neoadjuvant Therapy in Esophageal Adenocarcinoma[J]. Clinical Cancer Research, 2022, 28(12): 2669-2678.
[11] Jin Y, Zhao X, Song X, et al. The High Expression of p53 Is Predictive of Poor Survival Rather TP53 Mutation in Esophageal Squamous Cell Carcinoma[J]. Journal of Oncology, 2023, 2023.
[12] Gharaibeh L, Elmadany N, Alwosaibai K, et al. Notch1 in cancer therapy: possible clinical implications and challenges[J]. Molecular pharmacology, 2020, 98(5): 559-576.
[13] Wu H, Yu Z, Liu Y, et al. Genomic characterization reveals distinct mutation landscapes and therapeutic implications in neuroendocrine carcinomas of the gastrointestinal tract[J]. Cancer Communications, 2022, 42(12): 1367-1386.
[14] Abby E, Dentro S C, Hall M W, et al. Notch1 mutations drive clonal expansion in normal esophageal epithelium but impair tumor growth[J]. Nature Genetics, 2023, 55(2): 232-245.
[15] Liu M, Liu Y, Zhou R, et al. Absence of NOTCH1 mutation and presence of CDKN2A deletion predict progression of esophageal lesions[J]. The Journal of Pathology, 2022, 258(1): 38-48.
[16] Song Z, Liu Y, Cheng G, et al. Distinct mutational backgrounds and clonal architectures implicated prognostic discrepancies in small-cell carcinomas of the esophagus and lung[J]. Cell Death & Disease, 2021, 12(5): 472.
[17] Liang J, Wang Y, Cai L, et al. Comparative genomic analysis reveals genetic variations in multiple primary esophageal squamous cell carcinoma of chinese population[J]. Frontiers in Oncology, 2022, 12: 868301.
[18] Vega M E, Giroux V, Natsuizaka M, et al. Inhibition of Notch signaling enhances transdifferentiation of the esophageal squamous epithelium towards a Barrett's-like metaplasia via KLF4[J]. Cell Cycle, 2014, 13(24): 3857-3866.
[19] Natsugoe S, Xiangming C, Matsumoto M, et al. Smad4 and transforming growth factor β1 expression in patients with squamous cell carcinoma of the esophagus[J]. Clinical cancer research, 2002, 8(6): 1838-1842.
[20] Fukuchi M, Masuda N, Miyazaki T, et al. Decreased Smad4 expression in the transforming growth factor-β signaling pathway during progression of esophageal squamous cell carcinoma[J]. Cancer: Interdisciplinary International Journal of the American Cancer Society, 2002, 95(4): 737-743.
[21] Teng Y, Sun A-N, Pan X-C, et al. Synergistic function of Smad4 and PTEN in suppressing forestomach squamous cell carcinoma in the mouse[J]. Cancer research, 2006, 66(14): 6972-6981.
[22] Xu X, Zheng L, Hang N, et al. The transcription factor c-Jun regulates Smad4 expression by upregulating pre-miR-183 expression to promote invasion and metastasis of esophageal

squamous cell carcinomas[J]. In Vitro Cellular & Developmental Biology-Animal, 2020, 56: 550-558.

[23] Hu N, Wang C, Su H, et al. High frequency of CDKN2A alterations in esophageal squamous cell carcinoma from a high-risk Chinese population[J]. Genes, Chromosomes and Cancer, 2004, 39(3): 205-216.

[24] Müller L B, Meurer L, Lopes A B, et al. Stepwise expression of CDKN2A and RB1 proteins in esophageal mucosa from patients at high risk for squamous cell carcinoma[J]. Applied Immunohistochemistry & Molecular Morphology, 2014, 22(9): 669-673.

[25] Ghobadi N, Mehramiz M, Shahidsales S, et al. A genetic variant in CDKN2A/2B locus was associated with poor prognosis in patients with esophageal squamous cell carcinoma[J]. Journal of Cellular Physiology, 2019, 234(4): 5070-5076.

[26] Gao L, Guo R-Y, Lu H, et al. Clinical and Genomic Analysis of Patients with Short Survival after Surgery for Esophageal Squamous Cell Carcinoma[J]. Digestive Diseases, 2023, 41(3): 353-361.