Genetic Alterations of Esophageal Squamous Cell Carcinoma in Korean Patients

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Abstract

Background: Esophageal squamous cell carcinoma (ESCC) is one of the deadliest cancers in Korea, although its incidence is lower in Korea than in China and Japan. There are no data on genetic alterations associated with ESCC in Korea. Our study is the first report of the mutational landscape of ESCC in a Korean cohort.

Methods: We performed whole exome sequencing analysis of 43 ESCC tumor samples and germline DNA from normal esophageal mucosa or whole blood. Using electronic medical records, the patients' clinical characteristics were reviewed carefully.

Results: We found a mutually exclusive mutation pattern in *NFE2L2–ZNF750* gene pairs. Non-silent variants of *KMT2D* (*MLL2*) were associated with a poor prognosis. An analysis to identify potentially druggable genes revealed *NOTCH1* as a potential therapeutic target. We observed a high frequency of C:G → T:A transitions regardless of smoking history. In our cohort, deconstruction of the mutation signature revealed enrichment of COSMIC single base substitution (SBS) 13, SBS 39, SBS 2, SBS 40, and SBS 1.

Conclusion: We did not find a Korean-specific signature of genetic alterations, but our data suggest that *KMT2D* is a molecular prognostic marker. Further studies need to examine the role of *KMT2D* in ESCC prognosis due to our small sample size.

Introduction

Worldwide, esophageal cancer causes 544,076 deaths annually and is the sixth leading cause of cancer death.[1] East Asia has the highest regional incidence rates for both men and women, in part because of the large burden in China. In 2017, there were 232,255 new cases of cancer in Korea, of which 2483 (1.1% of all cancers) were esophageal cancer.[2] The crude incidence rate of esophageal cancer was 4.8 cases per 100,000 population in 2017. The age-standardized incidence rate decreased from 4.0 per 100,000 population in 1999 to 2.6 in 2017, with annual percent changes of −2.8% in men and −1.3% in women.[2] The incidence of esophageal cancer in Korea is relatively low compared with that in Japan and China, but the reasons for this are not clear.[3] In 2013, 90.2% of esophageal carcinoma cases was squamous cell carcinoma (ESCC) histologically, followed by adenocarcinoma (3%).[4] The proportions of localized and regional cancer tended to increase compared with that of distant cancer, and the 5-year relative survival rate of esophageal cancer improved from 14.0% in 1993–1995 to 38.0% in 2013–2017.[2] Nevertheless, the overall 5-year survival rate of patients with advanced cancer is less than 15%, and novel therapeutic targets are necessary for better treatment strategies for ESCC.[5]

Several studies have examined the genetic alterations associated with ESCC in Chinese, Japanese, and Indian populations.[5–10] For instance, a study of ESCC in a Chinese ESCC cohort of 139 reported known mutated genes, such as *TP53*, *PIK3CA*, and *NOTCH1*, and previously uncharacterized mutated genes including *FAT1*, *FAT2*, *ZNF750*, and *KMT2D*.[7] Song *et al*. reported mutual exclusivity between mutations

in *NOTCH1* and *PIK3CA*, and that patients with *NOTCH1* mutations had shorter survival times than patients without mutations.[10] In a whole exome sequencing (WES) analysis of 144 Japanese patients with ESCC, Sawada *et al*. identified mutations in *EP300* and *TET2* that correlated with a shorter survival time.[8] In a WES analysis of 28 Indians with ESCC, Kiran *et al*. reported higher frequencies of C:G → A:T transversion and *mutation signature 4* compared to smokers and non-users of tobacco.[5] However, the genomic alterations associated with ESCC in Korea are not well-characterized. In this study, we used WES to characterize the mutational landscape of ESCC in Korea.

Results

Results of whole exome sequencing analysis

Tumor and paired normal DNA from 43 ESCC patients was subjected to WES. The mean read depth of the target regions was 134.4⊆, and 99.0% of the target bases were covered by >10 independent reads after alignment. This identified 6,557 variants in 4,752 genes: 2,090 silent variants (synonymous and deep intronic variants) and 4,467 non-silent variants (Fig. 1). The non-silent variants include 3807 missense, 288 nonsense, 222 frameshift, 84 splicing, 51 in-frame, eight nonstop, and seven start codon variants. We found a median of 74 non-silent variants (range 18–307) per sample with a median tumor mutation burden of 2.47 (range 0.60–10.23) non-silent variants/MB.

Somatic mutation spectrum of esophageal squamous cell carcinoma

Our ESCC cohort included the recurrently mutated genes *TP53* (non-silent variants, 88% of samples), *TTN* (37%), *NFE2L2* (30%), *ZNF750* (23%), *NOTCH1* (21%), *FSIP2* (19%), *KMT2D* (16%), and *LRP1B* (16%) (Fig. 1, Supplementary Fig. S1). The top five affected oncogenic signaling pathways were *TP53* (88% of samples), *NOTCH* (58%), *RTK-RAS* (53%), *Hippo* (44%), and *NRF2* (37%) (Supplementary Fig. S2). We identified *NFE2L2* as a cancer driver gene with a single cluster, and all seven mutations were in that cluster based on the OncodriveCLUST algorithm run in maftools (p <0.05).[13] We performed Pfam annotation using maftools, and the top five mutated protein domains were COG5048, 7tm_1, FN3, Cadherin_repeat, and P53 (Supplementary Table S1). We observed a mutually exclusive mutation pattern in *NFE2L2*– *ZNF750* gene pairs in ESCC (Fig. 2). In our cohort, *NFE2L2* was mutated in 30% (13/43) of the samples and *ZNF750* in 23% (10/43) (Supplementary Fig. S1B, C).

Clinical and prognostic relevance of each mutation in esophageal squamous cell carcinoma

Table 1 summarizes the basic characteristics of the patients. The cohort contained 7 women and 35 men, with a median age of 70 (range 46–81) years. The median follow-up was 15.8 (range 2.3–31.4) months. Nodal metastasis was detected in 83.7% (7/43) of the patients and lympho-vascular invasion in 44.2%

(19/43), confirmed pathologically. All patients were followed until either death or the last follow-up date. During the follow-up period, 7 (16.3%) patients died and 13 (30.2%) suffered recurrence (Table 1).

In the clinical enrichment analysis, there were no genes significantly associated with sex, a history of alcohol or tobacco consumption, the grade of tumor differentiation, the grade of tumor invasion, the presence of nodal metastasis, lymphovascular invasion, or perineural invasion.

In the overall survival analysis of the top 30 genes, non-silent variants of *KMT2D* (*MLL2*) were associated with a poor prognosis (hazard ratio 6.18, $p < 0.05$) (Fig. 3A). *KMT2D* was mutated in 16.3% (7/43) of the samples in our cohort (Supplementary Fig. S1D). The mortality rates in the mutated and non-mutated *KMT2D* groups were 42.9% and 11.1%, respectively ($p = 0.072$). In the disease-free survival analysis of the top 30 genes, non-silent variants of *KMT2D* (*MLL2*) were associated with poor disease-free survival (hazard ratio 2.07) (Fig. 3B), but this was not significant ($p = 0.191$).

In the disease-free survival analysis of the top 30 genes, non-silent variants of *LRP1B* were associated with a greater likelihood of recurrence (hazard ratio 2.6), without statistical significance ($p = 0.083$) (Supplementary Fig. S3). The recurrence rates in the mutated and non-mutated *LRP1B* groups were 71.4% and 22.2%, respectively ($p < 0.05$). *LRP1B* was mutated in 16.3% (7/43) of the samples in our cohort, and all non-silent variants of *LRP1B* were missense variants (Supplementary Fig. S1E).

Table 1
Basic characteristics of patients with esophageal squamous cell carcinoma (ESCC) enrolled in this cohort.

Characteristic	Our cohort (n = 43) (%)
Sex	
Female	7 (16.3)
Male	36 (83.7)
Age (years)	
Range	46–81
Median ± standard deviation	65.88 ± 8.93
Degree of differentiation	
Well differentiated	7 (16.3)
Moderately differentiated	25 (58.1)
Poorly differentiated	11 (25.6)
Depth of invasion	
pT1b	8 (18.6)
pT2	9 (20.9)
pT3	24 (55.8)
pT4b	2 (4.7)
Nodal metastasis	
Yes	36 (83.7)
No	7 (16.3)
Lymphovascular invasion	
Yes	19 (44.2)
No	24 (55.8)
Death	
Yes	7 (16.3)
No	36 (83.7)
Recurrence	
Yes	13 (30.2)

Characteristic	Our cohort (n = 43) (%)
No	30 (69.8)
Alcohol consumption	
Current drinker	13 (30.2)
Ex-drinker	23 (53.5)
Non-drinker	7 (16.3)
Smoking status	
Current smoker	13 (30.2)
Ex-smoker	22 (51.2)
Non-smoker	8 (18.6)

Identification of potentially druggable genes

To identify potential druggable targets, we screened genes bearing non-silent variants detected in at least 20% of the samples against FDA-approved antineoplastic drugs in DGIdb.[14] This revealed *NOTCH1* as a potential therapeutic target. In our cohort, *NOTCH1* was mutated in 21% (9/43) of the samples (Supplementary Fig. S1F).

Mutation signatures associated with smoking history

In this study, seven samples were from non-smokers. The median mutation burden was 2.47 and 2.43 non-silent variants/MB in smokers and non-smokers, respectively. We observed a high frequency of C:G → T:A transitions regardless of smoking history. Further, deconstruction of the mutation signature of our cohort revealed the enrichment of COSMIC single base substitution (SBS) 13, SBS 39, SBS 2, SBS 40, and SBS 1 (Fig. 4). We did not observe distinct signatures associated with ESCC in patients with a history of smoking or alcohol consumption.

Discussion

This study investigated the mutational landscape of ESCC in a Korean cohort. We identified several known and novel variants in ESCC. The observed median tumor mutation burden of non-silent variants (2.47/MB) was comparable to the reported mutation load of 1.9–3/MB.[5–9,15] We identified several frequently mutated genes, such

as *TP53*, *TTN*, *NFE2L2*, *ZNF750*, *NOTCH1*, *FSIP2*, and *KMT2D*, which have been reported in ESCC. Mutated genes commonly found in over 10% of the ESCC samples in previous reports and this study are

described in Supplementary Table S2. Except for *TTN*, *NFE2L2*, and *FSIP2*, most of these genes had nonsense, frameshift, or splicing variants, suggesting their tumor suppressor roles (Fig. 1).

The NRF2 pathway consisting of *NFE2L2*, *KEAP1*, and *CUL3* was genetically deregulated in 37.2% of our cohort (Supplementary Fig. S1). *NFE2L2* encodes a transcription factor that induces the production of a cytoprotective enzyme in response to oxidative stress, whereas the KEAP1/CUL-dependent proteasomal mechanism degrades the NFE2L2 protein under non-stressed circumstances.[16] All *NFE2L2* variants in our cohort involved two hot spots within the DLG and ETGE motifs that bind to *KEAP1*, as observed in a Japanese cohort.[8] These mutations are thought to contribute to tumor development by stabilizing the NFE2L2 protein.[17] There were *NFE2L2* mutations in 9.6–16.7% of the cases in previous reports.[6,8,18] Recently, in a Chinese cohort of 508 patients, *NFE2L2* mutations were reported to be significantly associated with a worse prognosis of ESCC.[19] But there was no significant association between the prognosis of ESCC and *NFE2L2* mutations based on our data.

ZNF750, an epidermal differentiation regulator, is thought to be an ESCC tumor suppressor gene,[7,8,15] as supported by our data, which showed that most *ZNF750* variants were null variants (Fig. 1). *ZNF750* is mutated in 3.9–16.7% of ESCC cases.[6,18,20] In Japanese ESCC datasets, *ZNF750* variants were positively associated with the APOBEC signature,[8] but not in our study ($p = 0.069$).

We observed a mutually exclusive mutation pattern in *NFE2L2–ZNF750* gene pairs in ESCC, although this mutual exclusivity has not been implicated in any type of cancer.[21] Mutual exclusivity has been widely observed in cancer genomes and there are two major hypotheses associated with mutually exclusive mutated genes in cancers: the functional redundancy in downstream pathways and synthetic lethality hypotheses.[22] Because these two genes do not share the same pathway, the mutually exclusive mutation in *NFE2L2–ZNF750* gene pairs supports the synthetic lethality hypothesis.

NOTCH1 is frequently disrupted by loss-of-function mutations, implying that the loss of NOTCH pathway activity is critical for the growth of tumor cells with squamous differentiation characteristics.[23] *NOTCH1* is mutated in about 16% of cases.[6,8,18] There are several reports that *NOTCH1* is associated with a poor prognosis, and its mutations are mutually exclusive with *PIK3CA* mutations.[5,10,24] However, there was no significant association between the prognosis of ESCC and *NOTCH1* mutations based on our data. Nevertheless, the analysis to identify potentially druggable genes revealed *NOTCH1* as a potential therapeutic target.

KMT2D is tumor suppressor gene that encodes histone methyltransferase and promotes the transcriptional activation of target genes by modifying *histone H3 lysine 4 trimethylation* (H3K4me3).[25] *KMT2D* is mutated in 11–19% of ESCC cases,[6,8,18] and Kaplan–Meier survival analysis showed that patients with non-silent variants of *KMT2D* had poor overall survival. Abulajiang *et al*. reported an association between *KMT2D* expression and the prognosis of ESCC, with *KMT2D* overexpression predicting poor clinical outcomes and facilitating ESCC tumor progression.[26]

LRP1B is putative tumor suppressor gene that encodes low-density lipoprotein receptor-related protein 1b[27]. *LRP1B* is frequently deleted in various tumors and was deleted in 20.8% of a Japanese cohort[8] and mutated in 25% of an Indian cohort.[5] A study of *LRP1B* gene expression in ESCC showed that 42.9% of primary esophageal cancer cases have homozygous *LRP1B* deletions, and *LRP1B* mRNA expression was frequently silenced in cell lines without homozygous deletions (37.8%).[28] Bisulfite-PCR analysis and sequencing showed that *LRP1B*-nonexpressing cells without homozygous deletions were highly methylated at a *LRP1B* CpG island. Recently, Brown *et al*. reported better immune checkpoint inhibitor treatment responses in a group with likely pathogenic/pathogenic variants in *LRP1B* genes compared with a group carrying variant of unknown significance, indicating that mutations in the *LRP1B* gene have significant implications for the prognosis and treatment of multiple cancer types.[27] All *LRP1B* variants in our study were missense variants. Further studies of their expression and methylation are needed to clarify the roles of these *LRP1B* missense mutations.

Kiran *et al*. showed that tobacco chewers had a higher frequency of *mutation signature 4* than did smokers and non-users of tobacco in an Indian ESCC cohort.[5] There were no tobacco chewers in our study and no distinct signatures associated with ESCC in patients with a smoking history.

Our study is the first report of the mutational landscape of ESCC in a Korean cohort. It provides insight into molecular alterations in ESCC in Koreans and reveals potential candidates for therapeutic targeting. Our data suggest that *KMT2D* is a molecular prognostic marker. Further studies need to examine the role of *KMT2D* in ESCC prognosis due to the small size of our cohort. Additionally, copy number and expression studies of ESCC in Koreans are needed, as none were conducted here.

Conclusion

ESCC is one of the deadliest cancers in Korea, although its incidence in Korea is lower than in China and Japan. There are no data on genomic alterations associated with ESCC in Korea. Our study is the first report of the mutational landscape of ESCC in a Korean cohort. We found a mutually exclusive mutation pattern in NFE2L2–ZNF750 gene pairs. Non-silent variants of KMT2D (MLL2) were associated with a poor prognosis. An analysis to identify potentially druggable genes revealed NOTCH1 as a potential therapeutic target. We observed a high frequency of C:G → T:A transitions regardless of smoking history. Further, deconstruction of the mutation signature of our cohort revealed the enrichment of COSMIC SBS 13, SBS 39, SBS 2, SBS 40, and SBS 1. In summary, this study characterized the genomic alterations of ESCC with highly mutational heterogeneity, which provides the understanding of the molecular pathophysiology of ESCC, and it can be useful for finding potential therapeutic target in the aspect of precision medicine.

Materials And Methods

Patients and samples

We performed WES analysis of 43 ESCC tumor samples and germline DNA from normal esophageal mucosal tissue or whole blood obtained during surgical resection. The patients' clinical characteristics were reviewed using electronic medical records (Table 1). The study protocol was approved by the Institutional Review Board of Yonsei University College of Medicine (approval no. IRB 4-2018-1210). All subjects gave informed consent when they were enrolled and all methods were performed in accordance with the relevant guidelines and regulations of Institutional Review Board of Yonsei University College of Medicine.

Whole exome sequencing analysis

A WES DNA library was constructed using an Agilent SureSelect V6-Post kit (Agilent Technologies, Santa Clara, CA) with genomic DNA extracted from fresh-frozen tissue using the Qiagen DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany). Genomic DNA was sheared into 150–200-bp fragments using an ultrasonic sonicator. These sheared fragments were end-repaired, phosphorylated, adenylated at the 3' ends, and ligated using paired-end adaptors. The hybridization reaction was done at 65℃ for 16 hours using a DNA library with added biotinylated RNA library bait (Agilent Technologies). Cluster amplification was performed according to the manufacturer's protocol (Illumina, San Diego, CA). Paired-end sequencing was performed on an Illumina NovaSeq 6000 system (Illumina) by Macrogen Inc. (Seoul, South Korea).

Raw reads acquired in FASTQ format were trimmed using the Trimmomatic tool in paired-end mode to increase the mapping accuracy and specificity.[11] These were mapped against the reference genome hg19 (GRCh37) using the Burrows–Wheeler aligner with default parameters. Binary alignment map (bam) files were further processed using GATK (Genome Analysis Toolkit, Broad Institute, Cambridge, MA), which included the removal of duplicates using MarkDuplicates in Picard, IndelRealigner, and BaseRecalibrator. High-confidence somatic single-nucleotide variants (SNVs) were filtered using HaplotypeCaller, Mutect2, VarScan, and Pindel and annotated using AnnoVar and VEP. The variant call format (vcf) files were converted into mutation annotation format (maf) files using vcf2maf tools [Cyriac Kandoth (2020); mskcc/vcf2maf: vcf2maf v1.6.19, doi:10.5281/zenodo.593251].

Oncoplot, lollipop plots of somatic SNVs, the identities of genes with mutually exclusive variants and cancer driver genes, and actionable therapeutic target and mutational signatures were generated using the R package maftools.[12] To predict clinically actionable therapeutic targets, the Drug–Gene Interaction Database (DGIdb) was accessed, and the "Druggable Genome" category was used to identify potentially druggable targets in ESCC.[5]

Statistical analysis

The overall and disease-free survival distributions were described with Kaplan–Meier curves, and statistical significance was calculated using the log-rank test in STATA 16. Overall survival was evaluated from the time of diagnosis to death or the last follow-up. Censored cases were defined as patients who lost contact during follow-up and who were still alive at the end of the study. Disease-free survival was

evaluated from the time of diagnosis to death, the recurrence of ESCC, or the last follow-up. Censored cases were defined as patients who were still alive and had no recurrent tumors at the end of the study.

Clinical enrichment analysis was conducted using the R package maftools, with which pairwise and group-wise Fisher's exact tests can be performed to find differentially enriched genes for clinical features such as sex and determine whether metastasis has occurred, the patient survives, or the cancer has recurred.

Declarations

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

S.P analyzed, curated, interpreted the data, and wrote the paper. S.Y.P and S.T.L conceptualized this research, review, editing this paper, and supervised entire project. S.Y.P acquired the funding. All authors reviewed the results and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

References

1. Sung, H. *et al*. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca Cancer J Clin* **71**, 209–249 (2021).
2. Hong, S. *et al*. Cancer statistics in Korea: Incidence, mortality, survival, and prevalence in 2017. *Cancer Res Treat* **52**, 335–350 (2020).
3. Kim, D. J., Park, S. Y. & Hong, M. H. Esophageal squamous cell carcinoma, diagnosis and treatment. 363–376 (2020) doi:10.1007/978-981-15-4190-2_19.

4. Shin, A. *et al*. Trends in incidence and survival of esophageal cancer in Korea: Analysis of the Korea Central Cancer Registry Database. *J Gastroen Hepatol* **33**, 1961–1968 (2018).
5. Mangalaparthi, K. K. *et al*. Mutational landscape of esophageal squamous cell carcinoma in an Indian cohort. *Frontiers Oncol* **10**, 1457 (2020).
6. Gao, Y.-B. *et al*. Genetic landscape of esophageal squamous cell carcinoma. *Nat Genet* **46**, 1097–1102 (2014).
7. Lin, D.-C. *et al*. Genomic and molecular characterization of esophageal squamous cell carcinoma. *Nat Genet* **46**, 467–473 (2014).
8. Sawada, G. *et al*. Genomic landscape of esophageal squamous cell carcinoma in a Japanese population. *Gastroenterology* **150**, 1171–1182 (2016).
9. Song, Y. *et al*. Identification of genomic alterations in oesophageal squamous cell cancer. *Nature* **509**, 91–95 (2014).
10. Song, B. *et al*. Mutually exclusive mutations in NOTCH1 and PIK3CA associated with clinical prognosis and chemotherapy responses of esophageal squamous cell carcinoma in China. *Oncotarget* **7**, 3599–3613 (2015).
11. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
12. Mayakonda, A., Lin, D.-C., Assenov, Y., Plass, C. & Koeffler, H. P. Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res* **28**, 1747–1756 (2018).
13. Tamborero, D., Gonzalez-Perez, A. & Lopez-Bigas, N. OncodriveCLUST: exploiting the positional clustering of somatic mutations to identify cancer genes. *Bioinformatics* **29**, 2238–2244 (2013).
14. Griffith, M. *et al*. DGIdb: mining the druggable genome. *Nat Methods* **10**, 1209–1210 (2013).
15. Cheng, C. *et al*. Genomic analyses reveal FAM84B and the NOTCH pathway are associated with the progression of esophageal squamous cell carcinoma. *GigaScience* **5**, 1 (2016).
16. Shibata, T. *et al*. Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. *Proc National Acad Sci* **105**, 13568–13573 (2008).
17. Sporn, M. B. & Liby, K. T. NRF2 and cancer: the good, the bad and the importance of context. *Nat Rev Cancer* **12**, 564–571 (2012).
18. Du, P. *et al*. Comprehensive genomic analysis of oesophageal squamous cell carcinoma reveals clinical relevance. *Sci Rep-uk* **7**, 15324 (2017).
19. Cui, Y. *et al*. Whole-genome sequencing of 508 patients identifies key molecular features associated with poor prognosis in esophageal squamous cell carcinoma. *Cell Res* **30**, 902–913 (2020).
20. Zhang, L. *et al*. Genomic analyses reveal mutational signatures and frequently altered genes in esophageal squamous cell carcinoma. *Am J Hum Genetics* **96**, 597–611 (2015).
21. Kandoth, C. *et al*. Mutational landscape and significance across 12 major cancer types. *Nature* **502**, 333–339 (2013).

22. Deng, Y. *et al*. Identifying mutual exclusivity across cancer genomes: computational approaches to discover genetic interaction and reveal tumor vulnerability. *Brief Bioinform* **20**, 254–266 (2017).
23. Ntziachristos, P., Lim, J. S., Sage, J. & Aifantis, I. From fly wings to targeted cancer therapies: A centennial for notch signaling. *Cancer Cell* **25**, 318–334 (2014).
24. Ogawa, R. *et al*. NOTCH1 expression predicts patient prognosis in esophageal squamous cell cancer. *Eur Surg Res* **51**, 101–107 (2014).
25. Kerimoglu, C. *et al*. Histone-methyltransferase MLL2 (KMT2B) is required for memory formation in mice. *J Neurosci* **33**, 3452–3464 (2013).
26. Abudureheman, A. *et al*. High MLL2 expression predicts poor prognosis and promotes tumor progression by inducing EMT in esophageal squamous cell carcinoma. *J Cancer Res Clin* **144**, 1025–1035 (2018).
27. Brown, L. C. *et al*. LRP1B mutations are associated with favorable outcomes to immune checkpoint inhibitors across multiple cancer types. *J Immunother Cancer* **9**, e001792 (2021).
28. Sonoda, I. *et al*. Frequent silencing of Low Density Lipoprotein Receptor-Related Protein 1B (LRP1B) expression by genetic and epigenetic mechanisms in esophageal squamous cell carcinoma. *Cancer Res* **64**, 3741–3747 (2004).

Figures

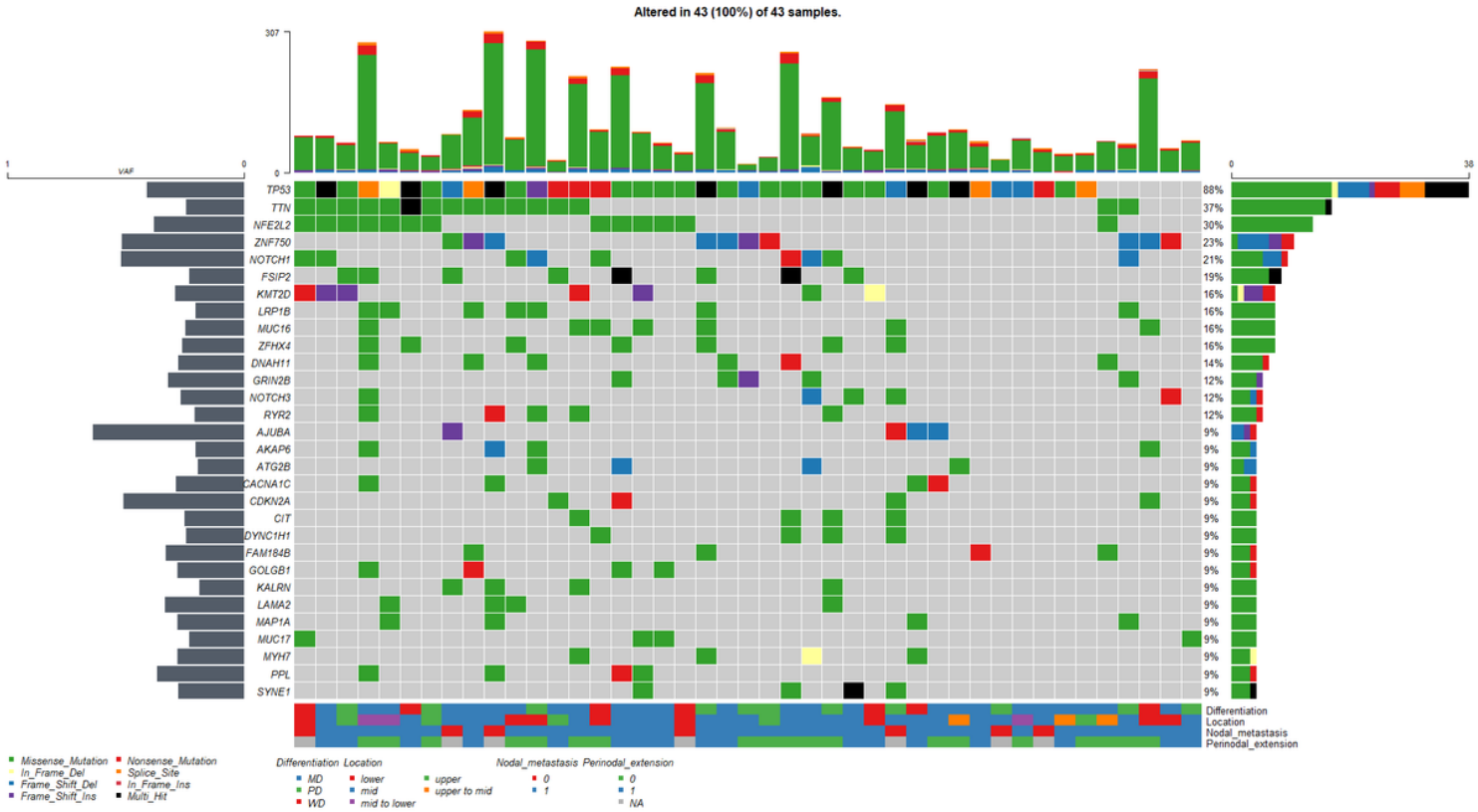

Figure 1

Oncoplots of the mutational landscape of esophageal squamous cell carcinoma (ESCC). The following characteristics of the top 30 genes are included: (top panel) the mutation load per MB, (middle panel) sample details including sample ID, tumor differentiation grade, tumor location, presence of nodal metastasis, and perinodal extension. Each column represents a sample and each row depicts a gene. Somatic mutations are colored according to the mutation type, and the right panel gives the percentages of samples harboring mutations.

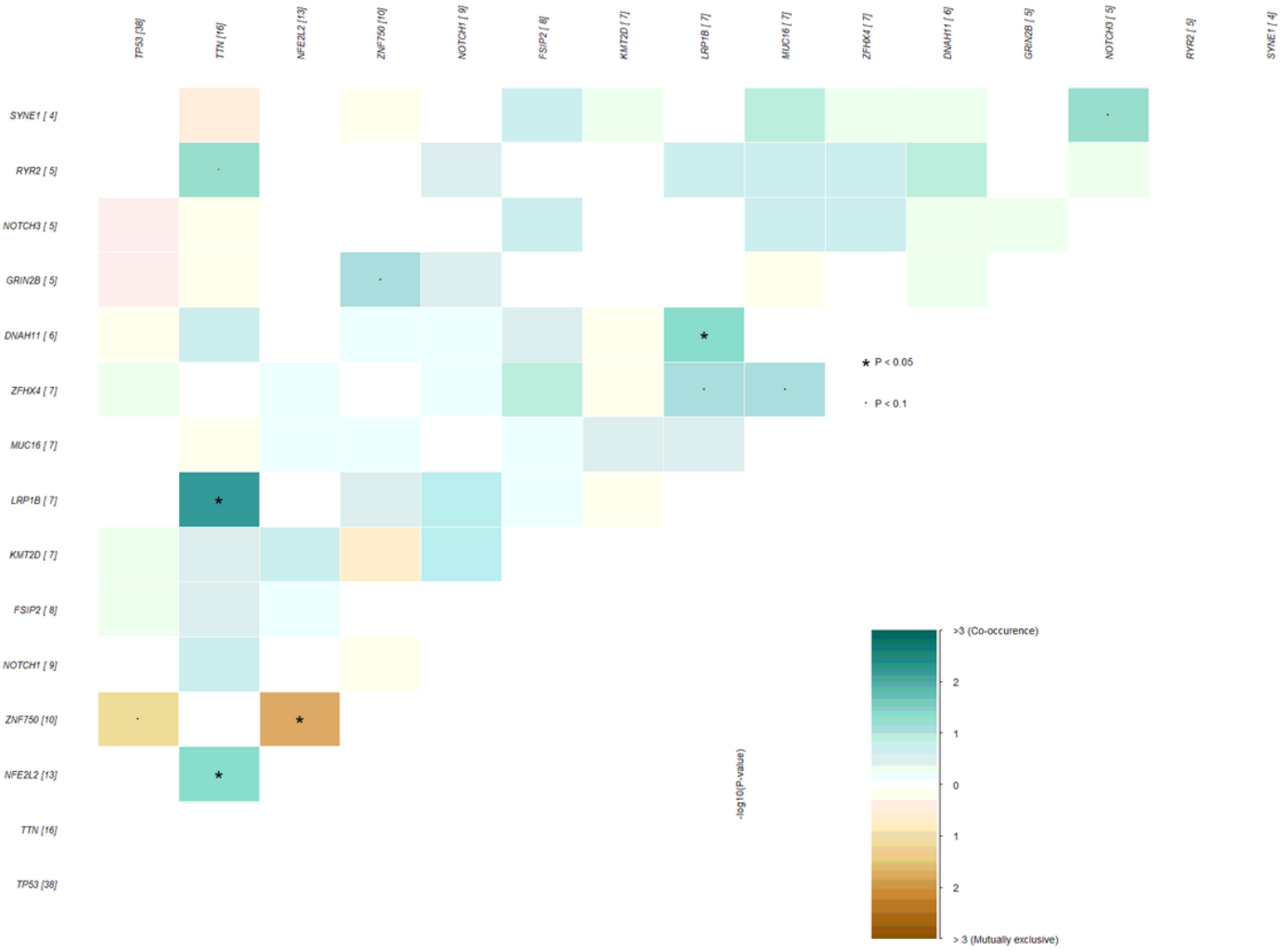

Figure 2

Somatic interactions of the top 15 genes in ESCC, using pair-wise Fisher's exact tests to detect significant gene pairs.

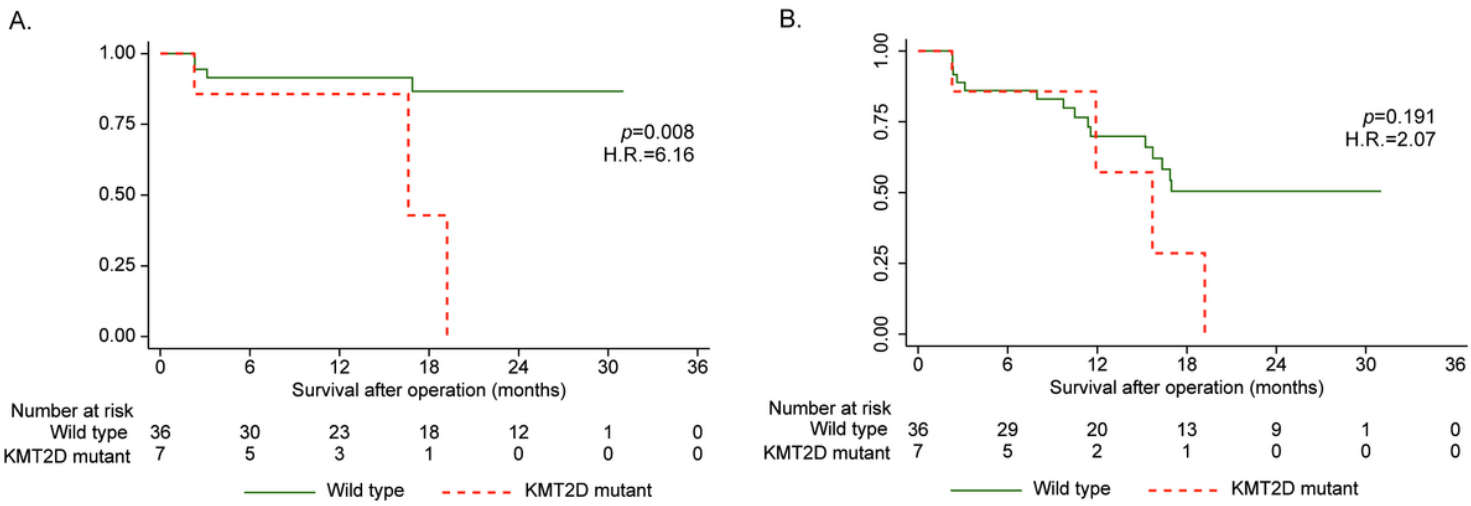

Figure 3

Overall survival (A) and recurrence (B) according to KMT2D mutation status. Kaplan–Meier survival curves of patients with KMT2D mutations or the wild type in our cohort. The p-values were computed using the log-rank test.

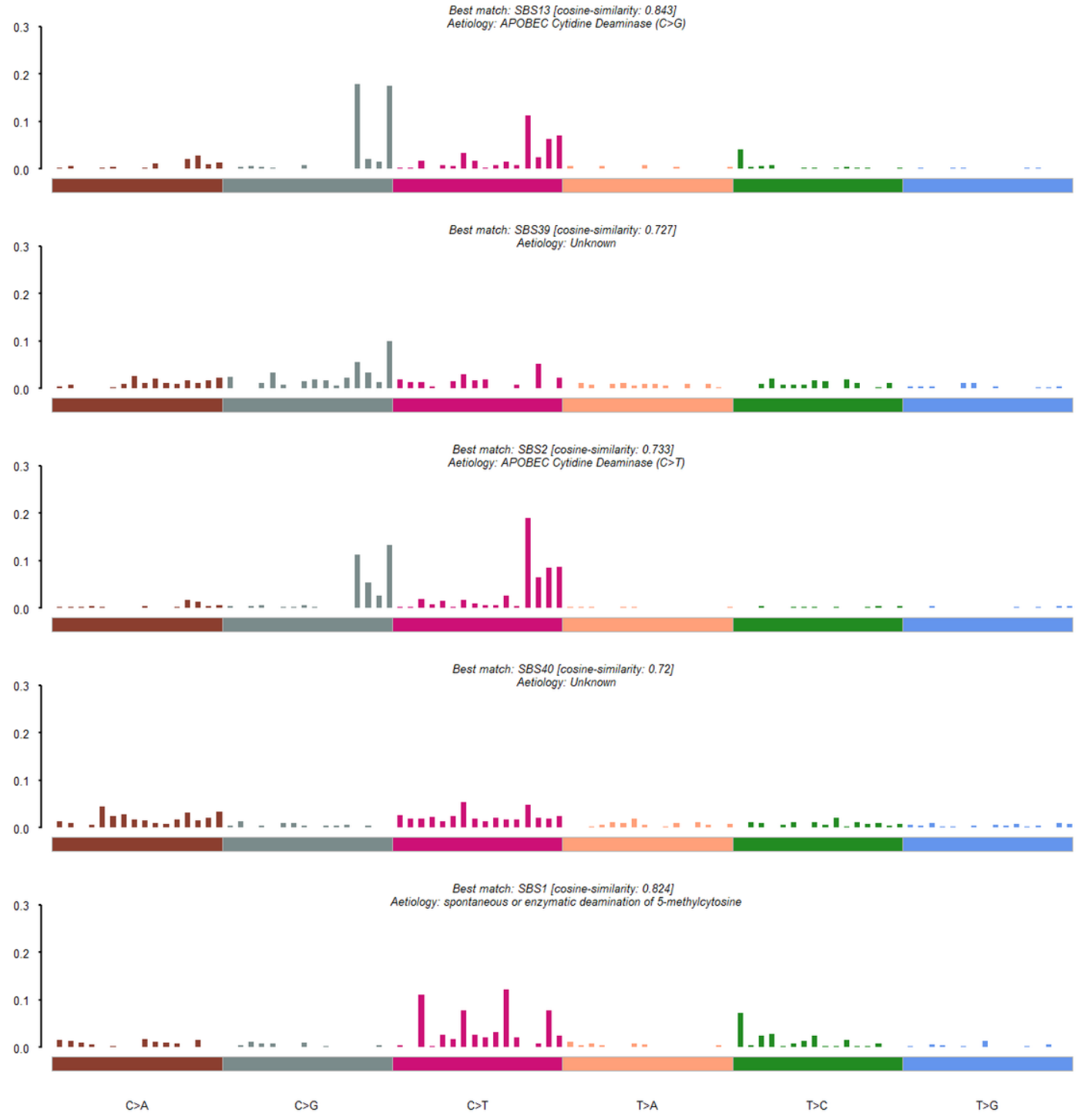

Figure 4

COSMIC single base substitution (SBS) signatures of our cohort.

Supplementary Files

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- Supplementalinfromation20211013.docx