ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 31(1): 22-27, 2026
DOI: 10.5505/ejm.2026.71224

Comprehensive Analysis of Co-Mutations in the PAPPA2 Gene in four Different Squamous Cell Carcinoma Types

Gülşah Evyapan[1*], Perçin Pazarcı[2]

[1]*Department of Medical Biology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey*
[2]*Department of Medical Biology, Faculty of Medicine, Cukurova University, Adana, Turkey*

ABSTRACT

The PAPPA2 mutation is one of the oncogenic mutations that interact with other cancer-causing mutations to promote tumour formation. Studying the presence of co-mutations in PAPPA2 gene could provide valuable prognostic and predictive information to help improve the therapeutic management of cancer treatment. In our study, we examined the co-mutation patterns of PAPPA2 in LUSC, HNSCC, ESCC and CSCC. Mutation data for LUSC, HNSC, ESCC, and CSCC were obtained from The Cancer Genome Atlas via cBioPortal. Data were analyzed using R (v4.4.3). For each cancer type, the 20 most frequently mutated genes were identified, and their co-occurrence with PAPPA2 was evaluated using Fisher's Exact Test ($p < 0.05$). Mutation landscapes were visualized using oncoprint plots. In LUSC, PAPPA2 mutations (18%) significantly co-occurred with SPTA1, RYR2, and TTN. Additional associations were found with LRP1B, MUC16, PKHD1, RYR3, and USH2A. In HNSC, PAPPA2 alterations (8%) showed strong co-occurrence with NSD1, PKHD1L1, and TP53, as well as with FLG, SYNE1, and CSMD3. In ESCC, PAPPA2 mutations (8%) co-occurred significantly with FMN2 and NFE2L2. In CSCC, PAPPA2 mutations (29%) exhibited extensive co-occurrence with DNAH5, USH2A, and APOB, along with several structural genes including COL11A1, HMCN1, and FAT4. No significant mutually exclusive relationships were identified in any cancer type. Oncoprint analyses demonstrated diverse mutation spectra, primarily composed of missense and multi-hit events. A better understanding of co-mutational profiles is crucial for improving oncology strategies and personalising treatment for SCC patients. The findings may be able to improve cancer prognosis and treatment if they are put into practice in a clinical setting.

Keywords: PAPPA2 mutations, Co-mutation analysis, TCGA, Cancer genomics

Introduction

Squamous cell carcinoma (SCC) is a common type of cancer that can develop in various organs, including the skin, lungs, lips, head and neck, bladder, stomach, vagina, and cervix. Around 1.8 million cases of SCC are diagnosed each year, with numbers having increased by up to 200 per cent over the past three decades(1). Each year, over 1 million people worldwide are affected by lung squamous cell carcinoma (LUSC), head and neck squamous cell carcinoma (HNSCC), oesophageal squamous cell carcinoma (ESCC) and cutaneous squamous cell carcinoma (CSCC)(2-5).

The PAPPA2 (pappalysin 2) gene, which encodes a member of the pappalysin family of metzincin metalloproteinases, is an important regulator of insulin-like growth factor (IGF) binding proteins (IGFBP). It interacts with IGFBPs to regulate IGF proteolysis and bioavailability(6). A number of functions are performed by IGFs, which include the regulation of cell proliferation, differentiation and survival. In addition, these peptides play a significant role in tumour formation(7). The interaction between growth factor binding proteins, extracellular matrix proteins and proteolytic enzymes constitutes the IGF-IGFR-IGFBP axis, which is crucial for tumor progression(8). Any changes to this delicate balance (e.g., the overexpression of an effector) can trigger a cascade of molecular events that can ultimately lead to malignancy. The development of cancer at various sites has been shown to be associated with abnormal IGF signalling. Evidence is growing that PAPPA2 is involved in the development of tumours and the spread of cancer to other parts of the body(9). However, the role of the PAPPA2 mutation in different types of cancer remains unclear and requires further investigation.

***Corresponding Author:** Gülşah Evyapan, Department of Medical Biology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey
E-mail: gulsahevyapan@yyu.edu.tr
ORCID ID: Gülşah Evyapan: 0000-0001-9870-4783, **Perçin Pazarcı:** 0000-0002-3708-0054
Received: 24.10.2025, **Accepted:** 03.12.2025

Current treatment methods still fail to benefit the majority of cancer patients due to the difficulty of identifying and prioritising targetable proteins on tumor cell surfaces. It is crucial to understand PAPPA2 mutations in SCC in order to develop personalised treatment strategies. Furthermore, because these mutations often coexist with other genetic alterations, identifying co-mutation patterns is a critical step in guiding treatment strategies for squamous cell carcinomas.

The simultaneous occurrence of multiple mutations within the same tumor, referred to as co-mutation or mutation co-occurrence, has gained growing interest as a means to identify cooperative genetic alterations or pathways that drive cancer development. Large-scale genomic resources such as The Cancer Genome Atlas (TCGA) have greatly advanced our understanding of cancer biology and gene regulation. TCGA offers comprehensive information on co-mutations and mutually exclusive mutations across numerous cancer types, supporting diverse studies ranging from tumor classification to the elucidation of oncogenic pathways. Our study aims to comprehensively investigate the co-mutation patterns of PAPPA2 in four different types of squamous cell cancer (HNSCC, LUSC, ESCC and CSCC) by analyzing the TCGA database.

Materials and Methods

This study investigated the co-mutation profile of the PAPPA2 gene across four distinct squamous cell carcinoma types: LUSC, HNSC, ESCC, and CSCC. All datasets were obtained from TCGA repository, which provides publicly accessible, high-quality genomic information from a wide range of human cancers.

Data Retrieval and Preparation: Mutation datasets for LUSC, HNSC, ESCC, and CSCC were accessed through the cBioPortal for Cancer Genomics(10), an open-access platform that provides harmonized TCGA mutation data and clinical annotations. This portal was used to download mutation profiles in Mutation Annotation Format (MAF) files, ensuring consistency with TCGA's genomic pipelines.

Mutation Profiling and Annotation: All retrieved mutation files were processed using R (v4.4.3). The maftools package was employed for mutation annotation and exploration, providing detailed information on variant classifications, affected genes, and mutation frequencies(11). For each cancer cohort, cases were divided into PAPPA2-mutant and wild-type groups to facilitate comparative assessment of genomic alterations.

Identification of Co-mutation Patterns: Within each squamous carcinoma dataset, the 20 most frequently mutated genes were determined using maftools functions. The statistical relationship between PAPPA2 and these recurrently mutated genes was then evaluated through Fisher's Exact Test. Co-occurrence and mutual exclusivity events were identified, with significance defined at $p < 0.05$.

Visualization and Statistical Analyses: To visualize the genomic interplay between PAPPA2 and other recurrently mutated genes, oncoprint diagrams were generated using the ComplexHeatmap package in R(12). These visualizations display mutation types and frequencies across individual samples, allowing clear identification of co-mutational trends and mutual exclusivity patterns. All statistical computations were performed within the R environment, utilizing relevant Bioconductor packages for reproducibility and data integrity. This workflow provides a robust framework for evaluating co-mutation signatures associated with PAPPA2 and facilitates cross-cancer comparisons of its molecular interactions.

Results

The mutation frequency of each gene was determined by counting them in patients with LUSC. Figure 1A shows the top 20 genes in the TCGA mutation database. The highest mutation frequency was observed among TP53, TTN, CSMD3, MUC16 and RYR2. In LUSC, PAPPA2 mutations (prevalence 18%) demonstrated significant co-occurrence with several genes. The strongest associations were with SPTA1 (p = 0.0000349, OR = 2.87), RYR2 (p = 0.000336, OR = 2.27), and TTN (p = 0.00125, OR = 2.37). Other significant co-occurring partners included LRP1B, MUC16, PKHD1, RYR3, and USH2A (Figure 1A). Oncoprint analysis revealed that PAPPA2 mutations are characterized by various mutation types, including missense and multi hit events (Figure 1B).

In HNSC, PAPPA2 alterations (prevalence 8%) showed strong co-occurrence, most significantly with NSD1 (p = 0.0000514, OR = 4.86), PKHD1L1 (p = 0.000785, OR = 4.10), and TP53 (p = 0.000863, OR = 4.74). Other notable associations were with FLG, SYNE1, XIRP2, CSMD3, TTN, PCLO, and MUC16 (Figure 2A). Oncoprint analysis demonstrated that

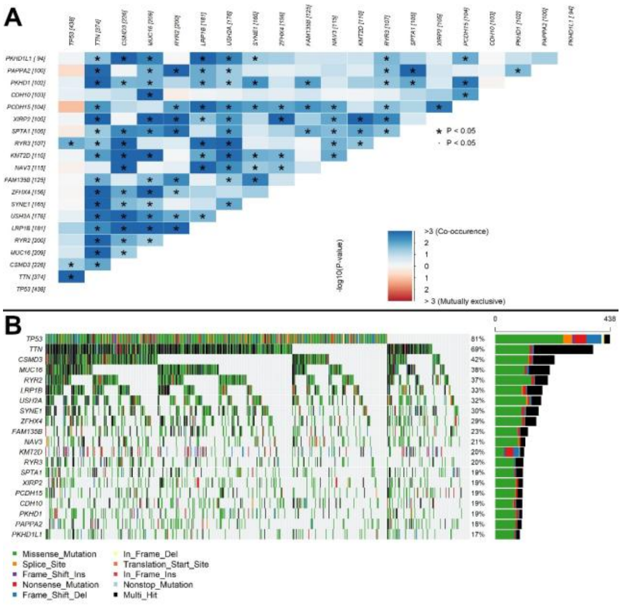

Fig. 1. Co-mutation and mutation profile of PAPPA2 in Lung Squamous Cell Carcinoma (LUSC) (n=544). (A) Co-mutation analysis of PAPPA2 (*: p < 0.05). (B) Oncoprint visualization of PAPPA2 and the top 20 mutated genes

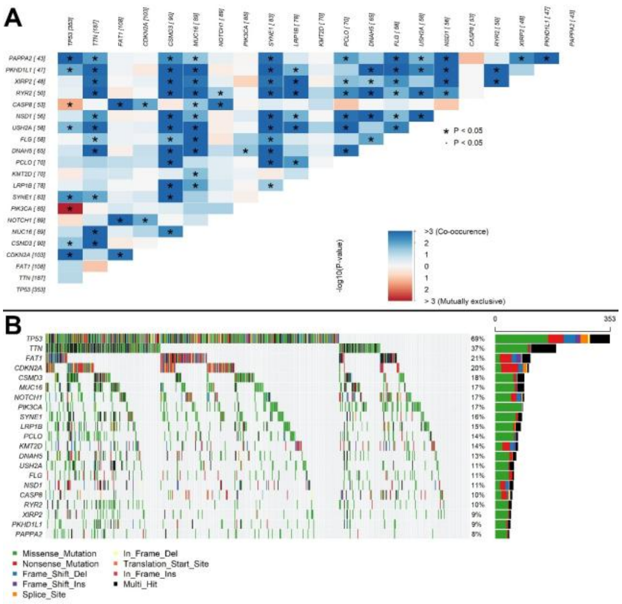

Fig. 2. Co-mutation and mutation profile of PAPPA2 in Head and Neck Squamous Cell Carcinoma (HNSC) (n=510). (A) Co-mutation analysis of PAPPA2 (*: p < 0.05). (B) Oncoprint visualization of PAPPA2 and the top 20 mutated genes

PAPPA2 mutation spectrum is diverse, encompassing mostly missense and nonsense events (Figure 2B).

In ESCC, PAPPA2 mutations (prevalence 8%) were significantly co-mutated with FMN2 (p =

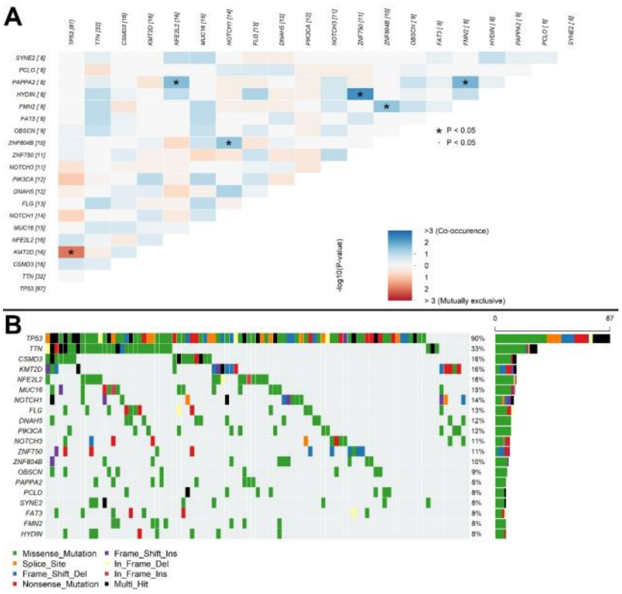

Fig. 3. Co-mutation and mutation profile of PAPPA2 in Esophageal Squamous Cell Carcinoma (ESCC) (n=97). (A) Co-mutation analysis of PAPPA2 (*: p < 0.05). (B) Oncoprint visualization of PAPPA2 and the top 20 mutated genes

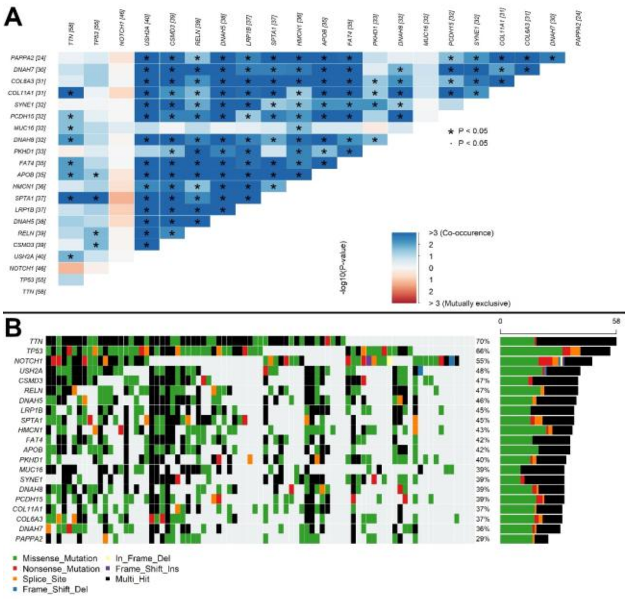

Fig. 4. Co-mutation and mutation profile of PAPPA2 in Cutaneous Squamous Cell Carcinoma (CSCC) (n=83). (A) Co-mutation analysis of PAPPA2 (*: p < 0.05). (B) Oncoprint visualization of PAPPA2 and the top 20 mutated genes

0.0173, OR = 9.60) and NFE2L2 (p = 0.0237, OR = 6.23) (Figure 3A). Oncoprint analysis revealed that PAPPA2 mutations are only missense mutations (Figure 3B).

Using TCGA datasets, we screened genes with high mutation rates linked to CSCC. In CSCC, PAPPA2 mutations (prevalence 29%) exhibited extensive co-occurrence, most strongly with DNAH5 ($p = 1.29\times10^{-6}$, OR = 16.62), USH2A ($p = 5.64\times10^{-6}$, OR = 14.22), and APOB ($p = 1.91\times10^{-5}$, OR = 9.88). Numerous other significant associations were identified, including SPTA1, COL11A1, COL6A3, HMCN1, FAT4, CSMD3, DNAH7, LRP1B, SYNE2, PCDH15, and RELN (Figure 4A). Oncoprint analysis shown that PAPPA2 mutations are characterized by missense and multi hit events (Figure 4B).

No significant mutually exclusive relationships ($p < 0.05$) were identified for PAPPA2 in any of the cancer types studied. Comutation analysis data is given as supplementary file.

Discussion

Although many studies on somatic mutations focus on the effect of individual mutations, researchers have recently started to pay more attention to the combined effects of multiple mutations occurring simultaneously in a tumor. Numerous studies suggest that co-mutations are a key determinant of oncogene-driven cancers, with these mutations occurring when different genes in a cell become altered at the same time(13).

SCC accounts for 90-95% of all cancer types(14). Furthermore, although squamous cell carcinoma has a lower incidence rate than adenocarcinoma, it has a lower cure rate(15). The clinical management and prognosis of SCCs have generally been unsatisfactory to date, making the urgent need for effective therapeutic strategies to reduce mortality a necessity. Advances in metrology technology, such as TCGA worldwide, have enabled us to obtain large amounts of data that reveal the genetic changes caused by SCC progression. This facilitates the identification of gene targets for the early diagnosis, treatment and prognosis of the disease. In the present study, we analysed the common mutation status of PAPPA2 in different cancer types.

Mutations in PAPPA2 frequently co-occur with alterations in other genes in SCCs. These co-mutations play a pivotal role in shaping tumor biology and influencing therapeutic responses. Significant co-mutations include TP53, TTN and MUC16 rearrangements. The coexistence of these mutations with PAPPA2 alterations in SCCs may contribute to enhanced tumor aggressiveness and increased resistance to therapeutic interventions.

Intracellular Ca^{2+} release channels are comprised of a number of components, one of which is RYR2. The role of RYR2 in calcium regulation is important(16). In this study, we demonstrated a significant co-mutation relationship between PAPPA2 and RYR2 in LUSC patients. This high co-mutation relationship of PAPPA2 suggests that PAPPA2 may play a role in processes related to calcium regulation. Currently, research on TTN mutations in tumors is quite limited. Furthermore, as mutated products of the SPTA1, TTN and RYR2 genes are frequently expressed in LUSCs(15, 17), these mutations can be used in LUSC patients both in the prognosis of the disease and as new treatment targets.

Findings from the TCGA-HNSC dataset, based on the TCGA database, demonstrated that PAPPA2 co-mutates significantly with NSD1, PKHD1L1 and TP53. A relationship has been reported between PKHD1L1 and tumour progression and genetic modifications in recent studies(18). Additionally, the NSD1 gene plays a significant role in histone methyltransferase activity(19, 20). Taken together, these findings suggest that the high co-mutation rate of PAPPA2 could contribute to more aggressive tumour progression and epigenetic deregulation.

PAPPA2 co-mutations and oncoprint analyses in ESCC reveal a complex mutational landscape characterised by both concurrent and mutually exclusive relationships. The top 20 genes in the TCGA mutation database are shown in Figure 3. Among these, TP53, TTN, CSMD3, KMT2D and MUC16 showed the highest mutation frequency. Approximately 8% of the samples were found to have PAPPA2 mutations, which were all missense variants. Co-mutation analysis revealed that, among these genes, PAPPA2 significantly co-occurred with NFE2L2 and FMN2. The protein known as nuclear factor erythroid-2-related factor-2, or NRF2 (also called NFE2L2), plays a crucial role in regulating the body's antioxidant response. It also plays a significant role in tumour progression and prognosis in many types of cancer(21). formin-2 (FMN2) is a versatile actin-binding protein that regulates important cytoskeletal processes in various biological contexts, facilitating the movement of chromatin and repair factors following DNA damage. Our study suggests that the simultaneous presence of PAPPA2, NFE2L2 and FMN2 may be an important driver gene in tumor progression in patients with ESCC.

CSCC is the second most prevalent form of non-melanoma skin cancer in the word. Previous studies have demonstrated that genetic factors play a key role in the formation and development of melanoma and that genetic mutations are an important biomarker for melanoma. This study found that, in CSCC patients, the co-mutation rates of PAPPA2 were highest with DNAH5, USH2A and APOB, as determined through TCGA data Recent studies have shown that DNAH5, which encodes the microtubule-associated motor protein dynein, and apolipoproteins (APOs), which play a key role in lipid exchange and metabolism, can activate the ERK1/2 pathway and thereby mediate lung tumour formation and progression in many cancers(22, 23). Taking all these factors into account, the results of the co-mutation analysis suggest that there may be a synergistic effect between the above genes.

In summary, our study investigated the co-mutation patterns of the PAPPA2 gene in four different squamous carcinoma types. Concurrent genomic alterations in genes have emerged as fundamental principles of molecular diversity in squamous cell carcinoma types. Therefore, prior knowledge of co-mutations may provide more detailed information about squamous cell carcinoma biology; facilitate the development of improved clinical response prediction algorithms, and enable the prediction and prevention of acquired resistance.

Among the limitations of the study, as the analyses were entirely in silico, the results obtained reveal only possible correlations rather than causal relationships. Furthermore, these co-mutation relationships do not evaluate functional outcomes.

By reducing the cost of confirmatory experiments compared to traditional methods, the TCGA has provided valuable insights into the molecular processes of various cancers, offering unparalleled insight into tumour-type specificity. Future studies should focus on confirming these co-mutational associations experimentally and investigating their functional consequences. Doing so could improve cancer prognosis and treatment when these findings are translated into clinical practice.

Ethics Committee Approval: The authors do not include any clinical or experimental research data directly from human participants or animal subjects for this study. All data in this study were obtained from publicly available databases. The author of this article declares that the materials and methods used in their research did not require ethics committee approval and/or any special legal permission.

Conflict of Interest Statement: The author reports no conflicts of interest in this work.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they participated in designing, executing and analysing the paper.

References

1. Caudill J, Thomas JE, Burkhart CG. The risk of metastases from squamous cell carcinoma of the skin. International journal of dermatology 2023; 62: 483-486.
2. Sacconi A, Muti P, Pulito C, Urbani G, Allegretti M, Pellini R, et al. Immunosignatures associated with TP53 status and co-mutations classify prognostically head and neck cancer patients. Molecular cancer 2023; 22: 192.
3. Tian H, Wei R, Xiao C, Fan T, Che Y, Liu T, et al. Tumor-derived KLK8 predicts inferior survival and promotes an immune-suppressive tumor microenvironment in lung squamous cell carcinoma. BMC pulmonary medicine 2024; 24: 53.
4. Rentroia-Pacheco B, Tokez S, Bramer EM, Venables ZC, van de Werken HJG, Bellomo D, et al. Personalised decision making to predict absolute metastatic risk in cutaneous squamous cell carcinoma: development and validation of a clinico-pathological model. EClinicalMedicine 2023; 63: 102150.
5. Jin Y, Meng Q, Zhang B, Xie C, Chen X, Tian B, et al. Cancer-associated fibroblasts-derived exosomal miR-3656 promotes the development and progression of esophageal squamous cell carcinoma via the ACAP2/PI3K-AKT signaling pathway. International journal of biological sciences 2021; 17: 3689-3701.
6. Dong Y, Zhao L, Duan J, Bai H, Chen D, Li S, et al. PAPPA2 mutation as a novel indicator stratifying beneficiaries of immune checkpoint inhibitors in skin cutaneous melanoma and non-small cell lung cancer. Cell proliferation 2022; 55: e13283.
7. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet (London, England) 2004; 363: 1346-1353.
8. Yu S, Zheng S, Qiu J, Lin H, Yu X, Chen H, et al. Overexpression of metalloproteinase

PAPPA accelerates cancer progression and correlates with immune cell infiltration in gastric cancer: insights from bioinformatics and in vitro investigations. Cancer cell international 2025; 25: 38.

9. Pan H, Hanada S, Zhao J, Mao L, Ma MZ. Protein secretion is required for pregnancy-associated plasma protein-A to promote lung cancer growth in vivo. PloS one 2012; 7: e48799.
10. https://www.cbioportal.org/.
11. Mayakonda A, Lin DC, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome research 2018; 28: 1747-1756.
12. Gu Z. Complex heatmap visualization. iMeta 2022; 1 :e43.
13. Skoulidis F, Heymach JV. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. Nature reviews Cancer 2019; 19: 495-509.
14. González-Guevara MB, Linares-Vieyra C, Castro-García ME, Muñiz-Lino MA, Abaroa-Chauvet C, Bello-Torrejón F. [Oral squamous cell carcinoma. Case report and review of literature]. Revista medica del Instituto Mexicano del Seguro Social 2022; 60: 85-90.
15. Zou S, Ye J, Hu S, Wei Y, Xu J. Mutations in the TTN Gene are a Prognostic Factor for Patients with Lung Squamous Cell Carcinomas. International journal of general medicine 2022; 15: 19-31.
16. Chang Y, Roy S, Pan Z. Store-Operated Calcium Channels as Drug Target in Gastroesophageal Cancers. Frontiers in pharmacology 2021; 12: 668730.
17. Iwakawa R, Kohno T, Totoki Y, Shibata T, Tsuchihara K, Mimaki S, et al. Expression and clinical significance of genes frequently mutated in small cell lung cancers defined by whole exome/RNA sequencing. Carcinogenesis 2015; 36: 616-621.
18. Zheng C, Quan R, Xia EJ, Bhandari A, Zhang X. Original tumour suppressor gene polycystic kidney and hepatic disease 1-like 1 is associated with thyroid cancer cell progression. Oncology letters 2019; 18: 3227-3235.
19. Farhangdoost N, Horth C, Hu B, Bareke E, Chen X, Li Y, et al. Chromatin dysregulation associated with NSD1 mutation in head and neck squamous cell carcinoma. Cell reports 2021; 34: 108769.
20. Fujimoto M, Andrew M, Dauber A. Disorders caused by genetic defects associated with GH-dependent genes: PAPPA2 defects. Molecular and cellular endocrinology 2020; 518: 110967.
21. Xu X, Yang Y, Liu X, Cao N, Zhang P, Zhao S, et al. NFE2L2/KEAP1 Mutations Correlate with Higher Tumor Mutational Burden Value/PD-L1 Expression and Potentiate Improved Clinical Outcome with Immunotherapy. The oncologist 2020; 25: e955-e63.
22. Darwish NM, Al-Hail MK, Mohamed Y, Al Saady R, Mohsen S, Zar A, et al. The Role of Apolipoproteins in the Commonest Cancers: A Review. Cancers 2023; 15(23).
23. Li F, Fang Z, Zhang J, Li C, Liu H, Xia J, et al. Identification of TRA2B-DNAH5 fusion as a novel oncogenic driver in human lung squamous cell carcinoma. Cell research 2016; 26: 1149-1164.