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RESEARCH ARTICLE
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EXPLORING THE ROLE OF TP53 IN KIDNEY RENAL CLEAR CELL CARCINOMA (KIRC): EXPRESSION ANALYSIS, PROMOTER METHYLATION, AND SURVIVAL ANALYSIS AND MUTATIONAL ANALYSIS THROUGH BIOINFORMATICS TOOLS

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Abstract

In this study we focus on the expression, promoter methylation, mutation analysis and overall survival of MAPK1 gene in lung adenocarcinoma (LUAD) patients with the help of bioinformatics tools. Firstly the expression pattern of MAPK1 was analyzed in patient sample and compare with control group. The consequences showed that gene of interest are crucially down regulated in LUAD patient as compare to control group. Then to verify the result the expression of targeted gene was analyzed on the basis of other pathological attributes for example stages of life, sex, ethnicity and phases of cancer. Remarkable deviation of MAPK1 gene expression was detected in LAUD sample versus normal samples which emphasizes the pathological importance of gene expression in LUAD patients. When we analyzed the promoter methylation of MAPK1 gene in LUAD patients versus normal group a slight hypo methylation was found that shows the epigenetic regulation of target gene. Subsequently KM plotter tool was used to analyze the overall survival (OS) rate of patient with the

expression of MAPK1 gene in LUAD sample in contrast to normal samples and found that overall good survival with low expression of the target gene. Mutation analysis of MAPK1 gene in LUAD sample by cBioPortal disclose that little genetic variation cause little dis-functioning of the gene. MAPK1 gene analysis point up the urgency of target gene as therapeutic and prognostic indicator in treatment of LUAD patients.

Key words: MAPK1: Cancer: Biomarker: Prognosis: UALCAN: TCGMA: KM plotter: cBioPortal: LUAD: EGFR

Introduction

A collection of disorders known as cancers are characterized by aberrant cell proliferation that has the capacity to infiltrate or spread to other bodily regions[1, 2]. After heart disease, cancer ranks as the second most common cause of death worldwide. Globally, cancer took the lives of one in six people [3-6].Twenty million new cases of cancer were expected to be reported worldwide in 2022[7]. In comparison, benign tumors do not metastasize. Signs and symptoms that may be present include the following: a lump, unusual bleeding, protracted cough, unexplained weight loss, and altered bowel movements. Although cancer may be the cause of these symptoms; there are other possible explanations as well [2, 8]. More than 100 different forms of cancer afflict people[2].

The most prevalent kind of lung cancer is adenocarcinoma, and like other types, it is identified by unique cellular and molecular characteristics [9].To differentiate it from small cell lung cancer, which exhibits a distinct behavior and prognosis, it is categorized as one of numerous non-small cell lung cancers (NSCLC). Additional classifications for lung cancer include subtypes and variations [10]. The most frequent complaints from patients are dyspnea and a chronic cough. Adenocarcinoma is the most frequent type of lung cancer in Asian and younger women populations, and it is more common in people with a history of cigarette smoking [11].With a 5-year survival rate of only 10-15%, lung cancer is the second most frequent type of cancer and the primary cause of cancer-related fatalities [12]. The brain is the most common location for metastases in lung cancer patients, proceeds by the liver, adrenal glands, and bones. Rarely does it affect the ovary [13]. According to earlier reports, the majority of lung cancer metastases to the ovaries were caused by small cell carcinoma. It is uncommon for lung adenocarcinoma(LUAD) to spread to the ovary, and its frequency is unknown [14].

The protein kinase made up of serine-threonine kinases is called mitogen-activated protein kinase, or MAPK. In the course of biological evolution, it has undergone excessive conservation. It can independently transduce extracellular signals into cells to control the expression of related genes and participate in the physiological processes of cell division, invasion, metastasis, and proliferation without the aid of the second messenger signal transduction system. Many MAPK pathway enzymes have been shown to express abnormally in female reproductive tract malignancies, breast cancer, ovarian cancer, and cervical cancer, according to earlier studies[15, 16].

Additionally, a lot of chemotherapeutic medications have been used extensively in clinical practice through this channel. The majority of researchers thought that the MAPK signaling pathway was the malignancies' therapeutic focus. Studies mostly examined the growth and death of tumor cell [17]. However, the invasion and metastasis of tumor cells have not been thoroughly studied. The MAPK pathway's subfamily, MAPK1, is over expressed in follicular lymphoma [18]. Some studies also discovered that the activation of MAPK1 signaling pathway might considerably accelerate the formation of epithelial-mesenchymal transition (EMT) in cervical cancer cells [19]. The low expression of MAPK1 in the glioblastoma multiforme was found. It suggests that MAPK1 has the diverse activities in the different tumor tissues, which can promote and also block the cancer gene, merely fulfilling the role of promotion in the cervical cancer[20].Mechanistically unique network alleles, frequently in conjunction with weak oncogenes in the MAPK pathway, cause EGFR network oncogenesis [21]. The RNA immune precipitation (RIP) assay and the luciferase reporter gene assay

were used. The expression of miR-490-3p, hsa_circ_101237, and MAPK1 in tissues and cells was found using qRT-PCR. In both NSCLC tissues and cell lines, the study discovered that Hsa_circRNA_101237 expression was elevated [22].
Thus, the data suggests that MAPK1 is involved in LUAD. In this work, we examined the role of MAPK1 in LUAD using a variety of bioinformatics techniques. To assess MAPK1 expression in LUAD patients, the Cancer Genome Atlas (TCGA), Kaplan-Meier (KM) Plotter, cBioPortal, and UALCAN platform were used.

Material and Methods

Expression analysis of MAPK1 in LUAD

A popular database for cancer analysis is UALCAN [23, 24]. The UALCAN database and TCGA platform data sorting tools were utilized to compare MAPK1 expression in the normal and LUAD samples. UALCAN is widely available and especially beneficial for cancer analysis [25]. We also used the UALCAN database.

Survival analysis of MAPK1 in LUAD

The Kaplan Meier (KM) plotter is a popular tool for survival study analysis[26]. The primary growth pattern-based architectural classification showed a strong relationship with both overall survival (OS) and disease-free survival[27].Using a KM plotter, we evaluated the LUAD deregulation and its effect on the overall survival (OS) of LUAD patients. A user-friendly application called KM Plotter is utilized to research the prognostic significance of gene expression. This platform is frequently used to calculate the effect of a particular gene on the overall survival of LUAD patients.

Mutational analysis of MAPK1 gene

When conducting genetic studies on cancer, cBioPortal is a helpful and useful database.[28]. We were able to determine the functional significance of these pathways and checkpoints in relation to cancer due to this investigation[29].It makes it possible to research clinical pathways, genetic variations, and references in a variety of tumor forms. Using this database, we performed a mutational analysis of the MAPK1 gene in LUAD samples.

Promoter methylation analysis of MAPK1 in LUAD patients

We examined the methylation status of the MAPK1 promoter in LUAD by means of the UALCAN database. The UALCAN database is extensively utilized to assess and collect data regarding gene expression, promoter methylation of DNA, infection with viruses, and clinical features of the 31 distinct types of cancer patients.[30-32]. The information about the promotor methylation level of LUAD in relation to the individual's age, sex, race, and stage of cancer was also processed.

Results

MAPK1 expression analysis versus control sample:

Initially, we compared the expression of MAPK1 in LUAD patients to a control group. We found variations in MAPK1 expression between the tumor and control samples.
According to the UALCAN database, Figure 1 demonstrates that the MAPK1 gene expression is significantly downregulated in LUAD individuals, with a P-value < 0.05. The majority of people understand $P < 0.05$ to indicate that there is a fewer than 5% chance that the result was the result of chance and a greater than 95% chance that the result was accurate[33]. Consequently, it was established that MAPK1 had a part in the development of LUAD disease.

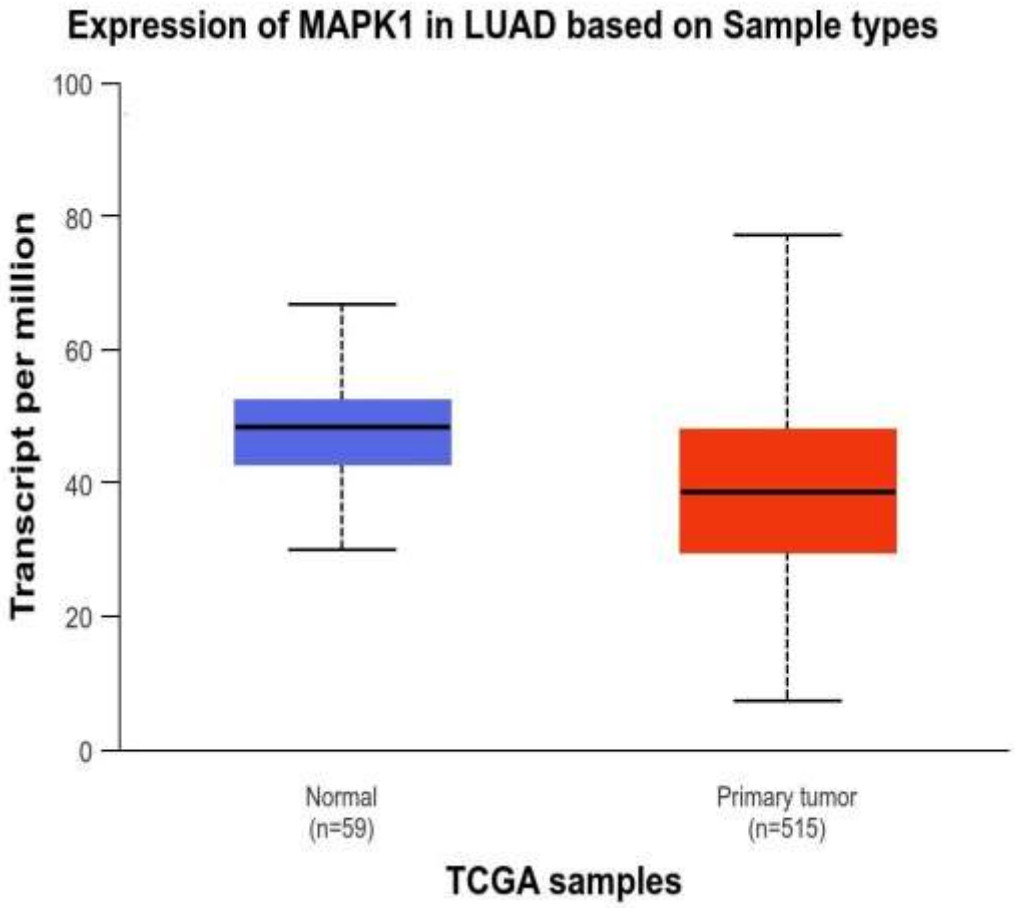

Figure 1: Shows that MAPK1 expression is down regulated in LUAD patients as compare to normal sample

On the basis of different parameters MAPK1 gene expression analysis in LUAD sample:

The target gene expression was analyzed in LUAD sample according to several parameters, such as the patient's gender, age, ethnic background, and tumor phase. First, we looked at the patient sample's MAPK1 expression based on different cancer stages. We looked at significant differences in MAPK1 expression at different phases of LUAD, as figure 2A illustrates. The degree of malignancy-related downregulation of MAPK1 decreases with increasing stage.

In the second graph, we look at MAPK1 expression in LUAD patients based on race. We examined the disparities in MAPK1 expression in the normal sample with the LUAD sample of various ethnic groups, as shown in figure 2C. Caucasians and African-American populations show more down regulation than Asian ones do.

In the third number, we compare the gender-based MAPK1 expression in the LUAD sample to the normal sample. In comparison to normal samples, Figure 2B shows that MAPK1 gene expression is significantly down regulated across male and female individuals with LUAD. Men experience a greater degree of down-regulation than do women.

Ultimately, we examined the levels of expression of the MAPK1 gene in patients with LUAD at various stages of life in comparison to normal individuals, and we observed significant variations in the MAPK1 gene expression samples between the two groups. As patients' ages increased, we saw a decrease in the degree of down regulation, as Figure 2D shows.

Thus, these studies revealed the importance of the MAPK1 gene in patients with LUAD.

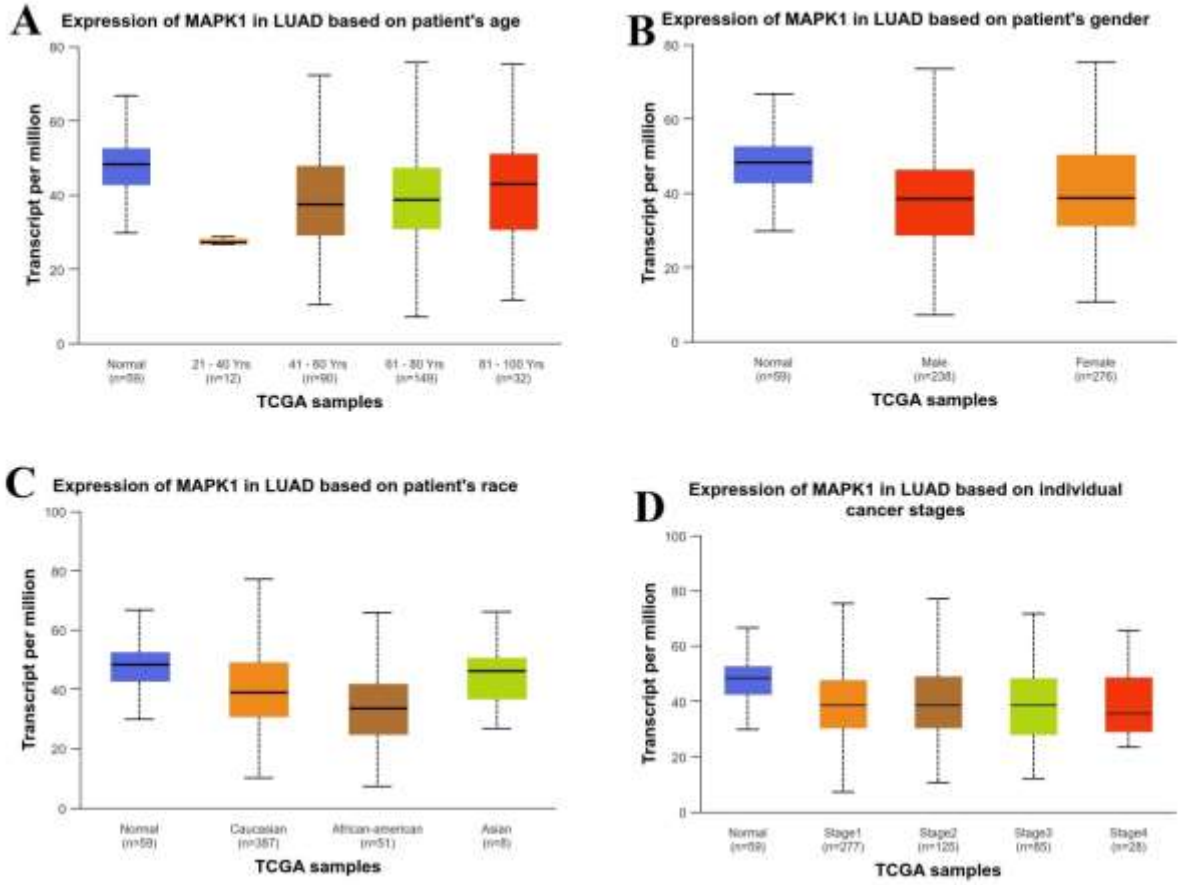

Figure 2: MAPK1 gene expression on the basis of different parameters

MAPK1 gene's promoter methylation in LUAD samples versus normal Samples

The UALCAN database was used to investigate methylation of the promoter of the MAPK1 gene in LUAD and control samples, as shown in figure 3. Previous research has shown the importance of promoter methylation on the expression of gene [34]. We found that the MAPK1 sample showed very less hypo-methylation compared to the untreated sample. Thus, in LUAD patients, there was variance in the expression of the MAPK1 gene, as indicated by this decreased promoter methylation.

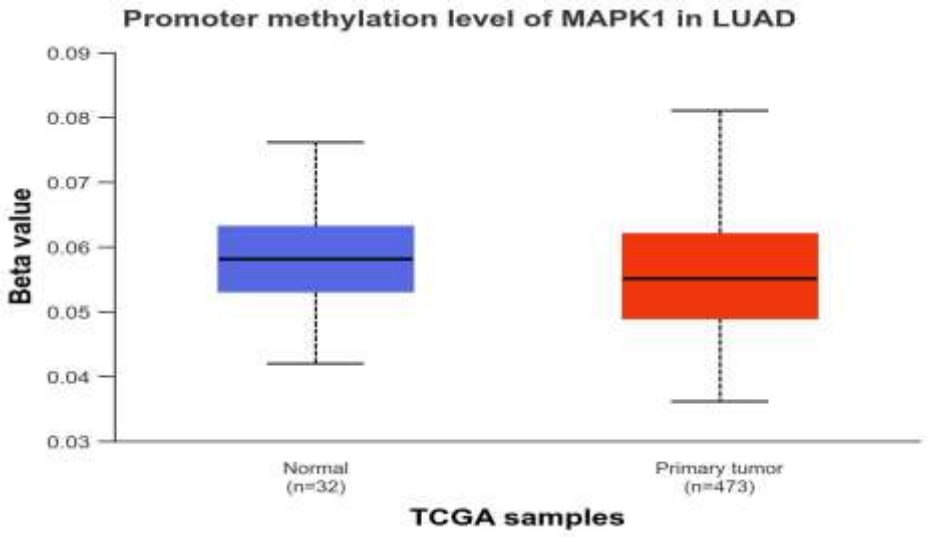

Figure 3: Promoter methylation profile of MAPK1 in LUAD patients versus normal samples

MAPK1 promoter methylation in LUAD sample based on different parameters:

The promoter methylation of MAPK1 in LUAD patients were analyzed on the basis of the patient's age, gender, race, and specific cancer stage. Initially, we looked at MAPK1 promoter methylation at different LUAD stages and found variations. While stage 1 and stage 2 of the LUAD exhibit the same amount of methylation as the control sample, we found significant hypomethylation at stages 4 (figure 4A). Next, we employed the race of the LUAD patients as a criterion to assess MAPK1 promotor methylation. We found that MAPK1 expression showed significantly low degree of hypomethylation in LUAD samples of African-American ethnicity compared to normal samples, and the Caucasian and Asian show high degree of hypomethylation as compare to the African-American group of patients shown in figure 4B. Next, we looked at the patient's gender-dependent MAPK1 promoter methylation levels in LUAD. We found that the MAPK1 showed hypomethylation as compare to control group, results also revealed that there was low degree hypo-methylation in the female as compare to male as shown in figure 4C. Next, we looked into the variation in MAPK1 promoter methylation levels in patient samples at different ages: patients aged 21–40, 41–60 and 61-80 years old showed hypo methylation, while patients aged 81–100 years old showed hyper methylation (figure 4D). So, our results imply that differences in MAPK1 expression could be caused by promotor methylation.

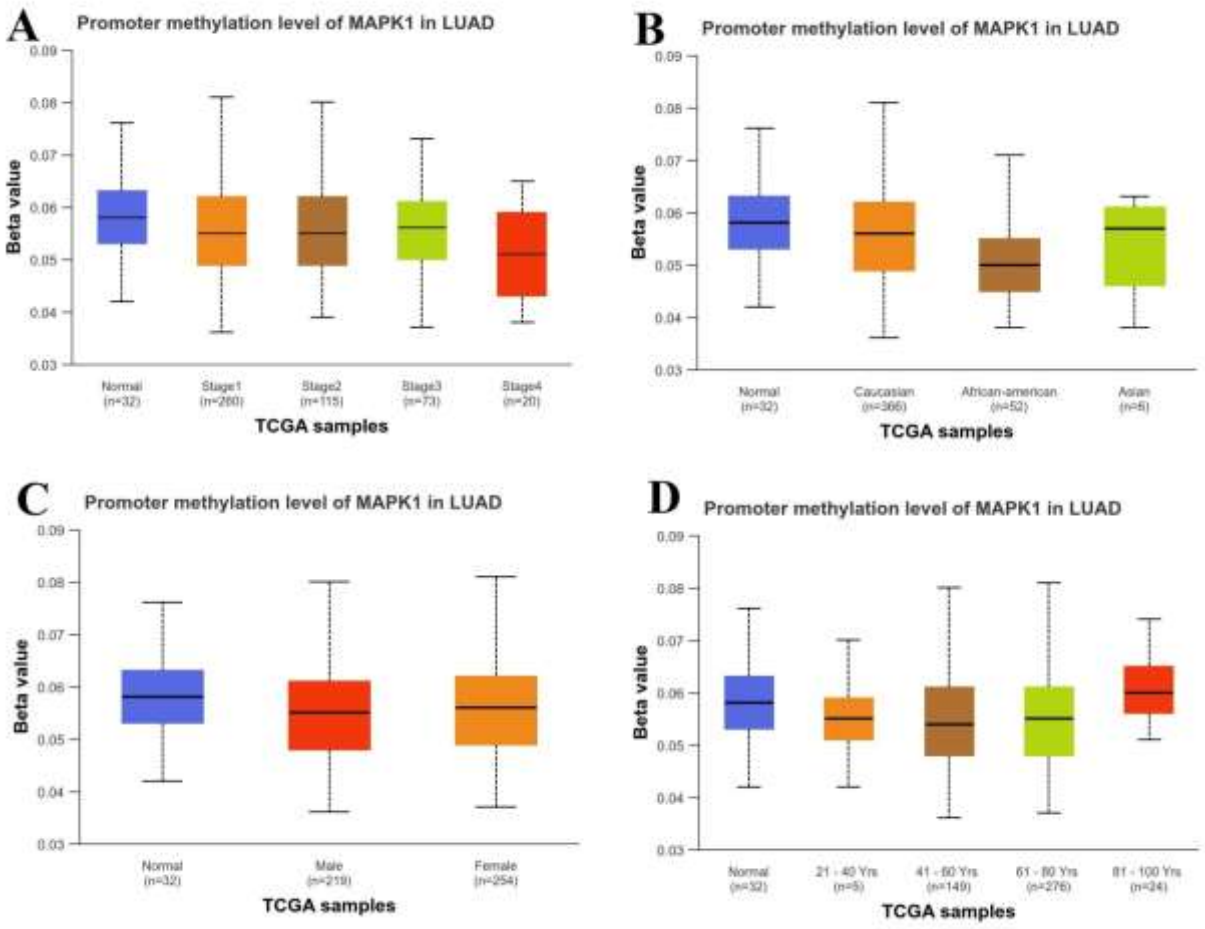

Figure 4: Promoter methylation profile of MAPK1 in LUAD samples versus normal samples on the basis of different pathological characteristics.

Survival analysis of MAPK1 in LUAD patients

We used the KM plotter tool to assess patient OS in order to ascertain the clinical significance of the MAPK1 gene in LUAD. We discovered that MAPK1 expression affects LUAD patient survival, as shown in Figure 5. If MAPK1 is downregulated, the patient exhibits overall favorable survival. While sufferers who had decreased MAPK1 expression had a considerably greater OS with p value 0.23,

which is more than 0.05 and indicates that the results are not significant, patients who had elevated MAPK1 expression had a low OS.

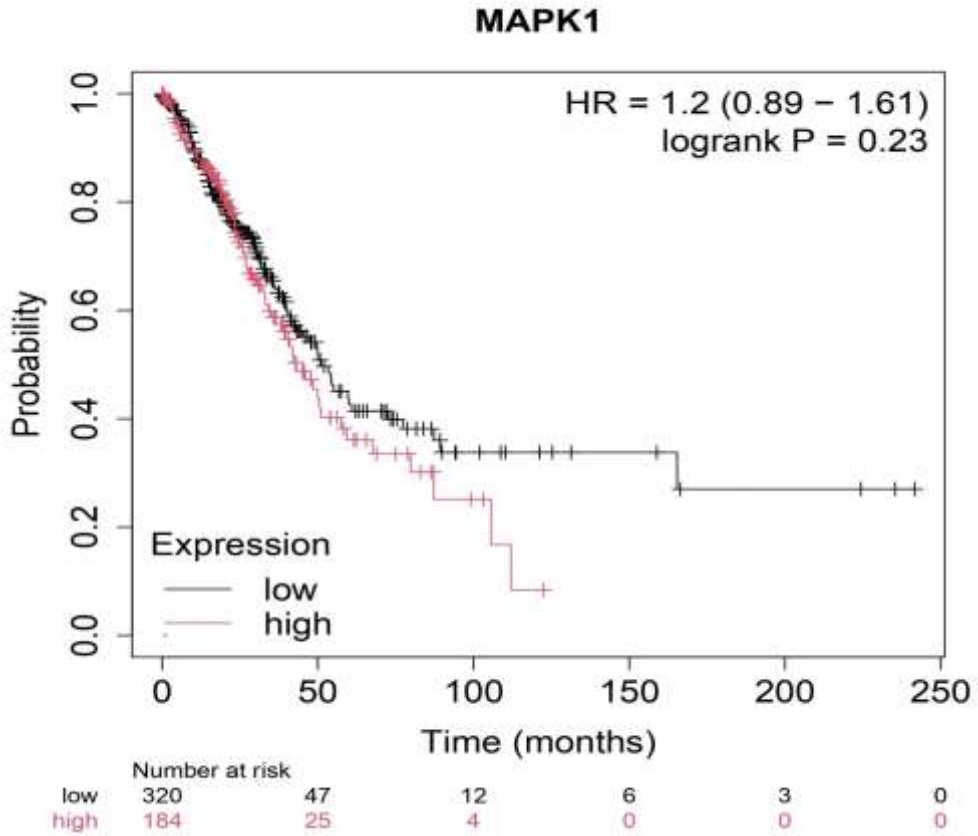

Figure 5: Overall Survival (OS) analysis of MAPK1 in LUAD samples

MAPK1 mutational analysis in LUAD

LUAD samples were subjected to MAPK1 mutational screening using the cBioPortal platform. We discovered a 1% mutation rate in MAPK1 in all LUAD samples.

This gene was shown to have two probable drivers for missense mutations, four missense mutations (of unknown relevance), zero truncating mutations (of unknown significance), nine amplifications, and one deep deletion, as shown in Figure 6.

Therefore, we investigated the possibility that a genetic mutation could affect MAPK1's function in LUAD in any way.

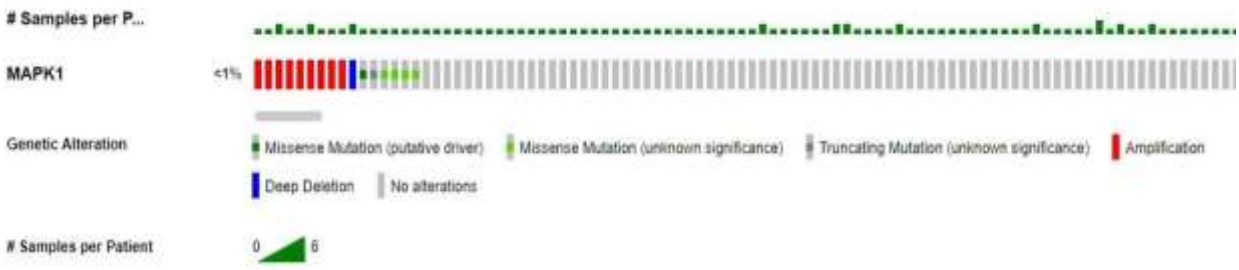

Figure 6: MAPK1 gene mutations analysis in LUAD patients

Discussion

We examined the expression, promoter methylation, survival assessment, and mutational study of the MAPK1 gene, which is clinically significant in lung adenocarcinoma (LUAD). Based on these investigations, we have made a number of important discoveries, which we compare and contrast with previous studies to assess the quality of our findings.

We initially looked at MAPK1 expression in the LUAD and control samples using the UALCAN database. In LUAD samples, MAPK1 expression was found to be down-regulated as opposed to normal samples; the significance level for this finding was 3.760E-3 as shown in figure 1. These data clearly show how important it is for LUAD expression. As a result, various ideas to prognostically classify lung adenocarcinomas based on differentiation have been put forth. A pattern-based classification approach was put forth in 2011[10]. It was reported that at stage 1, 2 and 3 MAPK1 gene expression was at equal rate but at stage 4 degree of down regulation is high. P value for stage 1, 3 and 4 are statistically significance but for stage 2 was non-significance because p value is more than 0.05 as shown in figure 2A. Significant racial and ethnic inequalities in the cancer burden were reported in a series of ACS reports released in the late 1980s[35]. It seems that socioeconomic factors such as insufficient health insurance, limited education and poverty were significantly more significant than biological variations[36, 37]. We found that there was high degree of down regulation of MAPK1 in African-american and P value was statistically significant as compare to the Caucasian and Asian LUAD patients as shown in figure 2C. Concern and risk perceptions are key concepts in many of the theoretical frameworks that are applied to the creation of cancer screening programs. Few studies have looked at gender differences because the majority of malignancies for which we have early detection or prevention measures are gender specific[38].The MAPK1 gene expression was analyzed on the basis of gender and down regulation of target gene expression was found and there P value was statistically significant as shown in figure 2B. When we analyzed the target gene expression on the basis of age we noticed that degree of down regulation is minimum for age group 81-100 years and there P value was not significant as shown in figure 2D. Age is one of the most researched risk factors for cancer and is measured by completed units of time. It is utilized in almost every investigation of cancer epidemiology. Since the probability of most malignancies rises with age, especially starting in midlife, cancer might be regarded as an age-related disease[39, 40].

We examined MAPK1 gene methylation in LUAD patients versus normal and discovered that there is hypo methylation of target gene and with non-significant P value which is 4.252E-01. One essential epigenetic modification that has a big impact on gene expression is promoter methylation[41, 42].Then to verify the results we examined the MAPK1 gene promoter methylation on the basis of different parameters which are given here (figure 3). We examined the MAPK1 gene methylation on the basis of cancer stage and found that at stage 1, 2, 3 are equally hypo methylated and at stage 4 the degree of hypomethylation is high (figure 4A). Then examined the MAPK1 gene promoter methylation in LUAD patients and found that hypomethylation of target gene as compare to normal and degree of hypo methylation is greater in African-american race as compare to Asian and Caucasian race, with significant P value 3.63E-02 (figure 4B). Subsequently MAPK1 gene promoter methylation was studied across the gender and found that results are not significant because P value is less than 0.05 (figure 4C). At the end we studied the Promoter methylation of target gene across the different age groups and noticed that first three groups showed hypomethylation and fourth age group showed significantly hypermethylation as shown in figure 4D.

As we conducted survival analysis, we also looked at the connection between the MAPK1 gene and overall survival (OS) in LUAD patients and found that overall good survival of the patient if the target gene is hypo regulated (figure 5). For mutational analysis, cBioPortal is also utilized. We found that less than 1% mutation rate was found in the MAPK1 gene in LUAD sample as shown in figure 6. We have determined the lifetime cancer risk linked to germline DNA mismatch repair gene mutations using based on populations approach, independent of a person's family history[43]. A Web-based tool for examining, displaying, and evaluating multimodal cancer genomics data is the cBioPortal for Cancer Genomics (http://cbioportal.org)[44]. Further research is necessary, although our results showed that genetic variations in LUAD patients had a minor effect on MAPK1 dysregulation.

Conclusion

In order to provide a thorough understanding of MAPK1 in patients with LUAD, our study focuses on promoter methylation status, expression models, predictive indicators, and mutation analysis.

These clarify how MAPK1 might work as a LUAD treatment and prognostic biomarker. Further study is needed to validate the data and set the stage for MAPK1's potential clinical applications in LUAD patients.

Conflict of interest
None

Acknowledgement
None

Reference

1. Dwivedi, M., A perspective review on cancer–the deadliest disease. International Journal of Cancer. **1**(1).
2. Heymach, J., et al., Clinical cancer advances 2018: annual report on progress against cancer from the American Society of Clinical Oncology. Journal of Clinical Oncology, 2018. **36**(10): p. 1020-1044.
3. Debela, D.T., et al., New approaches and procedures for cancer treatment: Current perspectives. SAGE open medicine, 2021. **9**: p. 20503121211034366.
4. Yasir, M., et al., Anti-bacterial activity of essential oils against multidrug-resistant foodborne pathogens isolated from raw milk. Brazilian Journal of Biology, 2022. **84**: p. e259449.
5. Hameed, Y. and S. Ejaz, TP53 lacks tetramerization and N-terminal domains due to novel inactivating mutations detected in leukemia patients. Journal of Cancer Research and Therapeutics, 2021. **17**(4): p. 931-937.
6. Usman, M., Y. Hameed, and M. Ahmad, Does human papillomavirus cause human colorectal cancer? Applying Bradford Hill criteria postulates. ecancermedicalscience, 2020. **14**.
7. Bray, F., et al., Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 2024. **74**(3): p. 229-263.
8. Nicholson, B.D., et al., The Suspected CANcer (SCAN) pathway: protocol for evaluating a new standard of care for patients with non-specific symptoms of cancer. BMJ open, 2018. **8**(1): p. e018168.
9. Travis, W.D., Pathology and genetics of tumours of the lung, pleura, thymus, and heart. (No Title), 2004: p. 344.
10. Travis, W.D., et al., International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. Journal of thoracic oncology, 2011. **6**(2): p. 244-285.
11. Herbst, R.S., D. Morgensztern, and C. Boshoff, The biology and management of non-small cell lung cancer. Nature, 2018. **553**(7689): p. 446-454.
12. Fujiwara, A., et al., Bilateral ovarian metastasis of non-small cell lung cancer with ALK rearrangement. Lung Cancer, 2014. **83**(2): p. 302-304.
13. Fujiwara, K., et al., Clinical implications of metastases to the ovary. Gynecologic oncology, 1995. **59**(1): p. 124-128.
14. Yada-Hashimoto, N., et al., Metastatic ovarian tumors: a review of 64 cases. Gynecologic oncology, 2003. **89**(2): p. 314-317.
15. Miller, P.C., et al., A novel mapk–microrna signature is predictive of hormone-therapy resistance and poor outcome in er-positive breast cancer. Clinical cancer research, 2015. **21**(2): p. 373-385.
16. Burotto, M., et al., The MAPK pathway across different malignancies: a new perspective. Cancer, 2014. **120**(22): p. 3446-3456.
17. Grossi, V., et al., p38α MAPK pathway: A key factor in colorectal cancer therapy and chemoresistance. World journal of gastroenterology: WJG, 2014. **20**(29): p. 9744.

18. Wang, W., et al., MicroRNA profiling of follicular lymphoma identifies microRNAs related to cell proliferation and tumor response. haematologica, 2012. **97**(4): p. 586.
19. Du, L., et al., CD44-positive cancer stem cells expressing cellular prion protein contribute to metastatic capacity in colorectal cancer. Cancer research, 2013. **73**(8): p. 2682-2694.
20. Erkutlu, I., et al., Correlation between Rho-kinase pathway gene expressions and development and progression of glioblastoma multiforme. Tumor Biology, 2013. **34**: p. 1139-1144.
21. Kaushik, S., et al., A tyrosine kinase protein interaction map reveals targetable EGFR network oncogenesis in lung cancer. bioRxiv, 2020: p. 2020.07.02.185173.
22. Zhang, Z.-y., et al., CircRNA_101237 promotes NSCLC progression via the miRNA-490-3p/MAPK1 axis. Scientific Reports, 2020. **10**(1): p. 9024.
23. Maciejczyk, A., et al., Elevated BUBR1 expression is associated with poor survival in early breast cancer patients: 15-year follow-up analysis. J Histochem Cytochem, 2013. **61**(5): p. 330-9.
24. Dong, Y., et al., Prognostic model development and molecular subtypes identification in bladder urothelial cancer by oxidative stress signatures. Aging, 2024. **16**(3): p. 2591-2616.
25. Chandrashekar, D.S., et al., UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia, 2017. **19**(8): p. 649-658.
26. Maciejczyk, A., et al., Elevated BUBR1 expression is associated with poor survival in early breast cancer patients: 15-year follow-up analysis. Journal of Histochemistry & Cytochemistry, 2013. **61**(5): p. 330-339.
27. Yoshizawa, A., et al., Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. Modern pathology, 2011. **24**(5): p. 653-664.
28. Cerami, E., et al., The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer discovery, 2012. **2**(5): p. 401-404.
29. Dong, Y., et al., Prognostic model development and molecular subtypes identification in bladder urothelial cancer by oxidative stress signatures. Aging (Albany NY), 2024. **16**(3): p. 2591.
30. Jia, B., et al., Prognostic roles of MAGE family members in breast cancer based on KM-Plotter Data. Oncol Lett, 2019. **18**(4): p. 3501-3516.
31. Hu, H., et al., CDCA8, a mitosis-related gene, as a prospective pan-cancer biomarker: implications for survival prognosis and oncogenic immunology. American Journal of Translational Research, 2024. **16**(2): p. 432.
32. Abdel-Maksoud, M.A., et al., Unlocking the diagnostic, prognostic roles, and immune implications of BAX gene expression in pan-cancer analysis. American Journal of Translational Research, 2024. **16**(1): p. 63.
33. Andrade, C., The P Value and Statistical Significance: Misunderstandings, Explanations, Challenges, and Alternatives. Indian Journal of Psychological Medicine, 2019. **41**(3): p. 210-215.
34. Yen, C.Y., et al., DNA methylation, histone acetylation and methylation of epigenetic modifications as a therapeutic approach for cancers. Cancer Lett, 2016. **373**(2): p. 185-92.
35. Society, A.C., Cancer and the poor: a report to the nation, 1989, American Cancer Society Atlanta, GA.
36. Freeman, H.P., Cancer in the socioeconomically disadvantaged. CA: A Cancer Journal for Clinicians, 1989. **39**(5): p. 266-288.
37. Freeman, H.P., Cancer in the economically disadvantaged. Cancer, 1989. **64**(S1): p. 324-334.
38. McQueen, A., et al., Risk perceptions and worry about cancer: does gender make a difference? Journal of health communication, 2008. **13**(1): p. 56-79.
39. WHO, G., International statistical classification of diseases and related health problems. 1992.
40. Group, U.C.S.W., United States cancer statistics: 1999–2010 incidence and mortality web-based report. Atlanta: US Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute, 2013. **201**.

41. Usman, M., et al., Breast cancer risk and human papillomavirus infection: a Bradford Hill criteria based evaluation. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders), 2022. **22**(4): p. 41-50.
42. Mao, J., et al., [Retracted] Risk Factors for TERT Promoter Mutations with Papillary Thyroid Carcinoma Patients: A Meta-Analysis and Systematic Review. Computational and Mathematical Methods in Medicine, 2022. **2022**(1): p. 1721526.
43. Dunlop, M.G., et al., Cancer Risk Associated with Germline DNA Mismatch Repair Gene Mutations. Human Molecular Genetics, 1997. **6**(1): p. 105-110.
44. Gao, J., et al., Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal. Science Signaling, 2013. **6**(269): p. pl1-pl1.