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EXPLORING THE ROLE OF ADENOMATOUS POLYPOSIS COLI IN HEAD AND NECK SQUAMOUS CELL CARCINOMA VIA MULTI-LEVEL IN SILICO METHODOLOGY

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Abstract
In this study we focused on the expression, promoter methylation, mutation analysis and overall survival of APC gene in Head and Neck Squamous cell Carcinoma (HNSC) patients. First of all, the expression pattern of APC gene was analyzed in HNSC patient sample as compared to control group by using UALCAN. Results of the analysis found remarkable down-regulation of APC gene expression in HNSC patients. When promoter methylation of APC gene was analyzed in HNSC patients versus normal group, a remarkable hypomethylation was found. Subsequently, KM plotter and GEPIA2 tools were used to analyze the overall survival (OS) of HNSC patient with respect to APC expression. Results showed that HNSC patients having higher expression of APC had shorter OS. Mutation analysis of APC gene in HNSC sample by cBioPortal discloses that minor genetic variations in APC gene. The correlation APC gene expression with other mutated genes in HNSC was evaluated using the mu-Target database. Results showed that mutations in GCNT$, AFG3L2, CCDC112, PRAGD and ADTRP genes may be associated by expression alteration in APC genes across HNSC patients. Finally, this study concluded that APC may be regarded as a unique prognostic biomarker for HNSC treatment.

Key words: UALCAN: TCGMA: KM plotter: cBioPortal: APC: HNSC: GEPIA2: mu Target

1 Introduction
The hallmark of cancer is the unchecked growth of cells that have escaped from central endogenous regulatory systems. Cancers are classified based on the organ or tissue from whence they originated, but more and more, this classification is also based on the molecular traits of the individual cancer

cells [1].One of the four main non-communicable diseases (NCDs) that account for about 14.6% of all fatalities in humans is cancer. There are already around 500 genes linked to cancer and over 100 distinct forms of cancer that are recognized. Research on the causes and treatments of cancer has been ongoing [2].The aberrant cell growth that sets apart a class of disorders known as malignancies and gives them the ability to invade and propagate to other parts of the body [3, 4].Cancer is the 2nd most prevalent cause of mortality globally, after cardiovascular disease. Cancer claimed the lives of one in six persons worldwide [5-8].It was anticipated that 20 million additional instances of cancer would be recorded globally in 2022 [9]. Possible signs and indicators include a lump, prolonged cough, unusual bleeding, unexplained weight loss, and changed bowel movements. These symptoms could be caused by cancer, but there are other reasons as well [4, 10]. It is believed that a subpopulation of potentially tumorigenic stem or stem-like cells causes and sustains cancers [11]. People suffer from more than 100 distinct types of cancer [4].

The term "head and neck cancer" refers to a broad category of malignancies that can arise in the head and neck area. These include tumors that affect the voice box (laryngeal), the throat, the salivary glands, the nose, the sinus cavities, and the mouth, tongue, gums, and oral carcinoma [12]. The symptoms of head and neck cancer can vary greatly, depending on the location of the tumor's origin. These can include a lump in the neck, voice changes, red or white spots in the mouth, an ulcer in the inside of the mouth that won't go away, and swallowing difficulties [13]. Head and neck squamous cell carcinoma (HNSC) is the collective term for the majority of head and neck malignancies, which originate from the mucosal epithelium of the oral cavity, pharynx, and larynx [14]. HNSC is the seventh most prevalent cancer worldwide, according to the most recent GLOBOCAN estimates (2020), which also show that it causes 450,000 annual fatalities (approximately 4.6% of all cancer deaths worldwide) and 890,000 new cases annually (about 4.5% of all cancer diagnoses worldwide) [15].

Testing for HPV (Human Papilloma Virus) status should be done on all neck node metastases of unknown cause and all squamous cell carcinomas originating from the oropharynx. This is necessary in order to correctly stage the tumor and formulate a treatment plan. Distinguishing between HPV positive and negative tumors is crucial for continuing research aimed at identifying the most effective treatments, as these malignancies differ biologically [16]. Cyfra 21-1, a tumor marker, could be helpful in the diagnosis of HNSC [17].

One category of gene for tumor suppressors is APC. Genes known as tumor suppressors stop cells from growing out of control, which could lead to malignant tumors. APC gene-produced protein is essential for a number of cellular processes that decide whether a cell will eventually become a tumor [18]. The Adenomatous Polyposis Coli (APC) is a gene that is commonly associated with mice (Apc), humans (APC), and both species' proteins (APC). APC is a big multifunctional protein that is often down-regulated or altered in malignancies that originate from epithelial cells [19, 20]. In humans, the APC gene is found in band q22.2 (5q22.2) on the long (q) arm of the 5th chromosome. It has been demonstrated that the APC gene possesses an internal ribosome entrance site [21]. There are 2,843 amino acids in the full-length of APC human protein, and its expected molecular mass is 311646 Da [22]. Metastasis is the primary cause of death in cases of oral squamous cell carcinomas (OSCC). Adenomatous polyposis coli (APC), catenins, and a family of glycoproteins known as cadherins are among the molecules that mediate intercellular adhesion [23]. Carriers of an APC-inactivating mutation have an almost 100% chance of developing colorectal cancer by the age of 40. However, mutations in β-catenin or APC must follow other alterations in order to become malignant [24]. In HNSC cell lines, the APC gene was also the most consistently hypermethylated gene [25].

Therefore, the evidence points to an APC gene involvement in HNSC. In this study, we used a range of bioinformatics methods to investigate the function of the APC gene in HNSC.

2. Material and Methods

2.1 Expression analysis of APC gene in HNSC

UALCAN is a well-known database for cancer investigation [26, 27]. The APC gene expression in the normal and HNSC samples was compared using the UALCAN database and the data sorting tools on the TCGA platform. UALCAN is a freely accessible tool that is particularly useful for cancer analysis [28]. The UALCAN database was also utilized for the analysis of APC gene expression.

2.2 Survival analysis of APC gene in HNSC

The Kaplan Meier (KM) plotter is a commonly used tool for survival study analysis [29]. We assessed the APC gene impact on the overall survival (OS) of patients with HNSC using a KM plotter.

2.3 Validation of APC gene expression and survival outcomes

The GEPIA2 database offers a reliable and in-depth analysis of TCGA data related to cancer [30]. To further validate the expression and survival outcomes of APC in HNSC, we employed GEPIA2. A statistically significant P value was defined as one less than 0.05.

2.4 Mutational analysis of APC gene

When doing genetic research on cancer, cBioPortal is a valuable resource [31]. This study allowed us to understand the practical importance of these pathways and regulators in connection to cancer [32]. More than 1% of all human genes are listed in the Cancer Gene Census (CGC) as having mutations that directly contribute to the development of cancer [33]. It enables the investigation of genetic variants, clinical pathways, and references in a range of tumor types. We carried out a mutational study of the APC gene in HNSC samples employing this database.

2.5 Promoter methylation analysis of APC in HNSC patients

Using the UALCAN database, we investigated the methylation status of the APC gene promoter in HNSC. The UALCAN database is widely used to evaluate information on the 31 different types of cancer patients' clinical characteristics, viral infection, promoter methylation of DNA, and gene expression [34-36].

Ethics

This study is purely based on bioinformatics analysis therefore no ethical approval was required

3. Results

3.1 APC gene expression analysis versus normal sample:

Firstly, we compared the expression of APC in patients with HNSC to a control group. Our findings showed that APC gene expression changed between tumor and control samples. The APC gene expression is down-regulated in HNSC patients, as shown in figure 1 of the UALCAN database; nevertheless, the results are not significant because the P-value is more than 0.05. A P value < 0.05 indicates a high probability of accuracy and a low probability of chance (37). As a result, it was revealed that APC contributed to the development of HNSC disease.

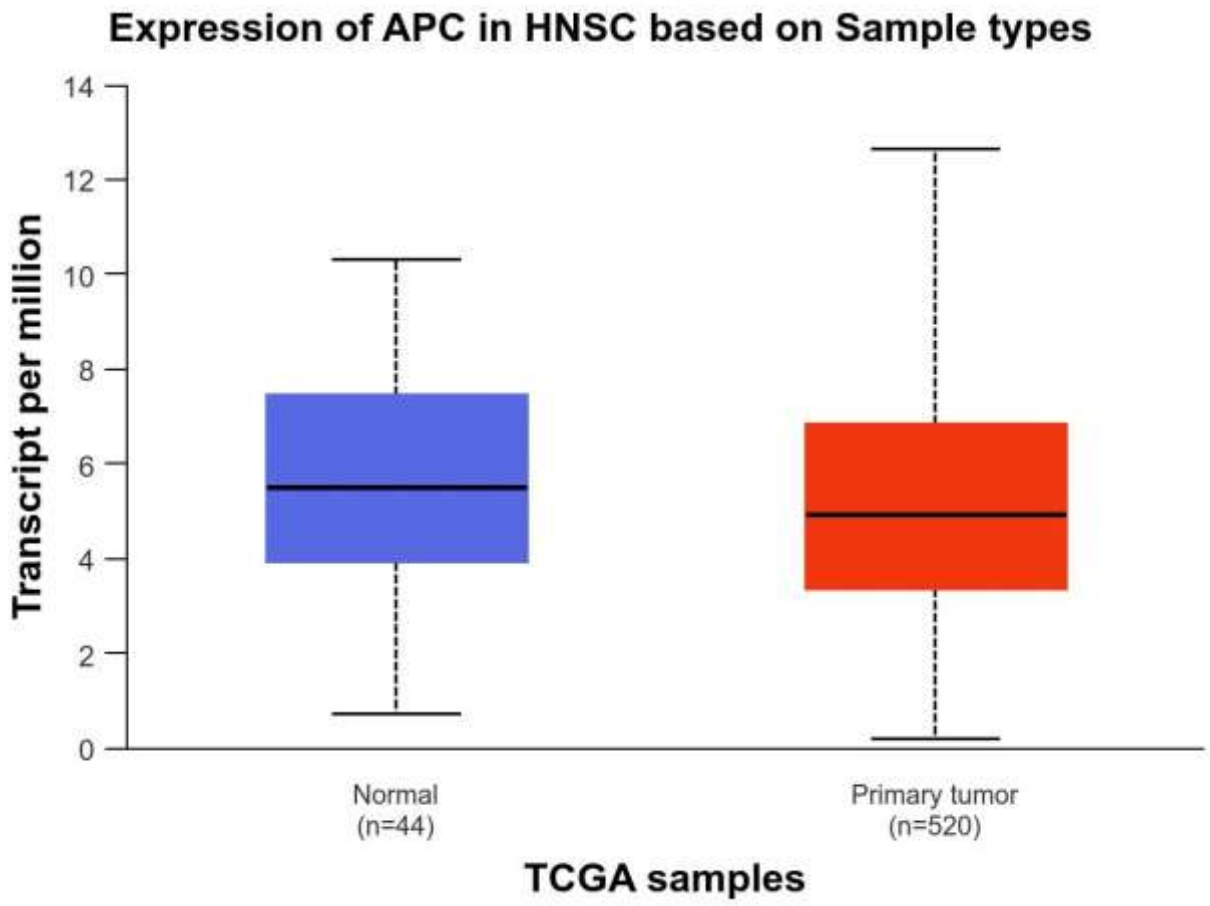

Figure 1: Expression of APC gene in HNSC and normal samples via the UALCAN. This figure shows that APC expression is down regulated in HNSC patients as compare to normal sample by using UALCAN.

3.2 APC gene expression in HNSC patients based on different parameters

Many criteria were considered while assessing APC gene expression in the HNSC sample, including the patient's gender, age, ethnic origin, and tumor phase. Figure 2A reveals that APC expression varies significantly at different stages of HNSC.As the stage progresses, so does the level of APC downregulation associated with cancer. The second graph compares APC expression in HNSC patients by race. Figure 2B shows that Asian populations have higher levels of downregulation than Caucasians and African-Americans.

The third graph compared gender-based APC expression in the HNSC sample and normal sample. Figure 2C shows that APC gene expression is significantly reduced in both male and female individuals with HNSC when compared to normal samples. In comparison to men, women are more down-regulated.

Finally, we compared the APC gene expression levels in HNSC patients at various age groups to those in healthy people, and we discovered significant variations in the APC gene expression samples between the two groups. As seen in Figure 2D, the degree of down regulation decreased with increasing patient age.

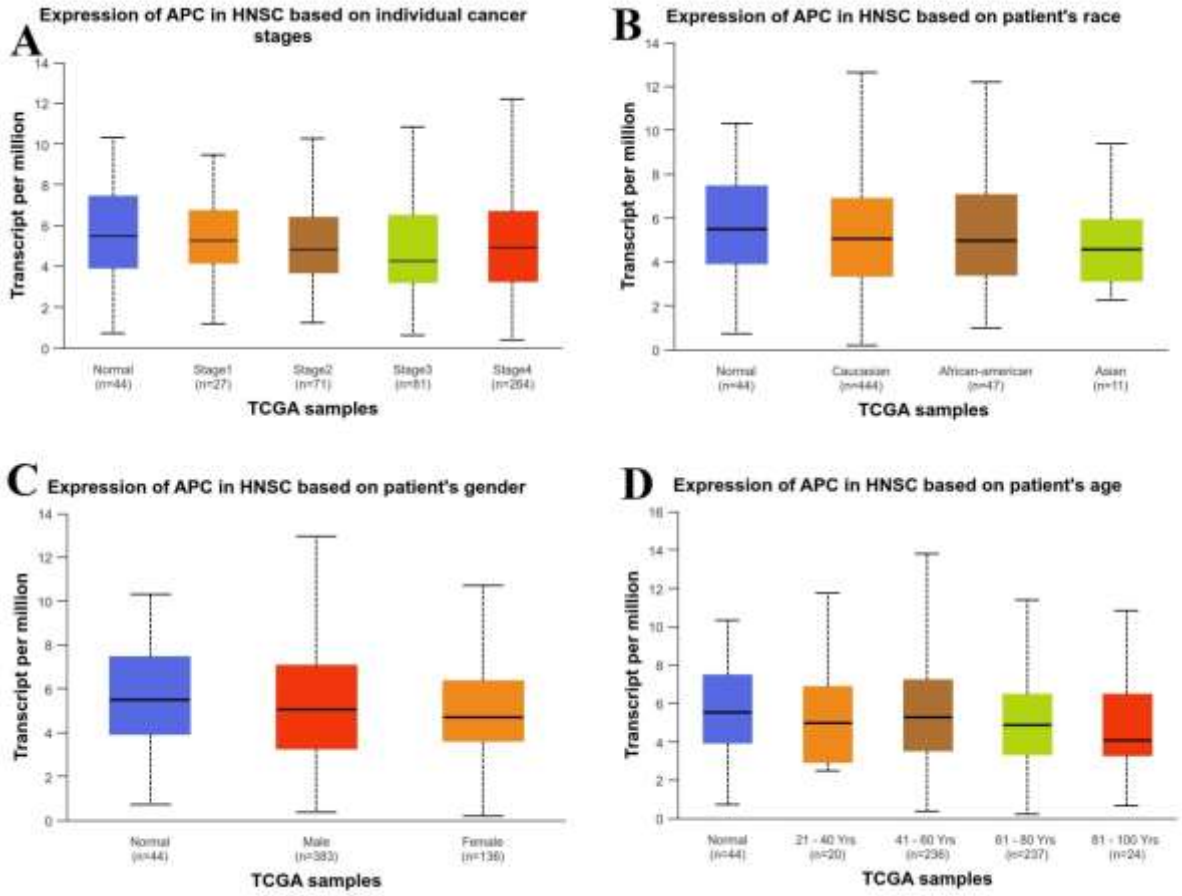

Figure 2: APC gene expression on the basis of different parameters. (A)Assessment of APC expression in HNSC stratified according to disease stages. (B) Assessment of APC expression in HNSC stratified according to ethinicity of patients. (C) Assessment of APC expression in HNSC stratified according to gender of patients. (D) Assessment of APC expression in HNSC stratifi ed according to patients age.

3.3 The promoter methylation of the APC gene is compared between normal and HNSC samples.

Prior research has revealed the importance of promoter methylation in gene expression (37). When compared to the control sample, we found that the APC sample had much less hypomethylation. This decreased promoter methylation shows that the expression of the APC gene varied between HNSC patients as shown in Figure 3.

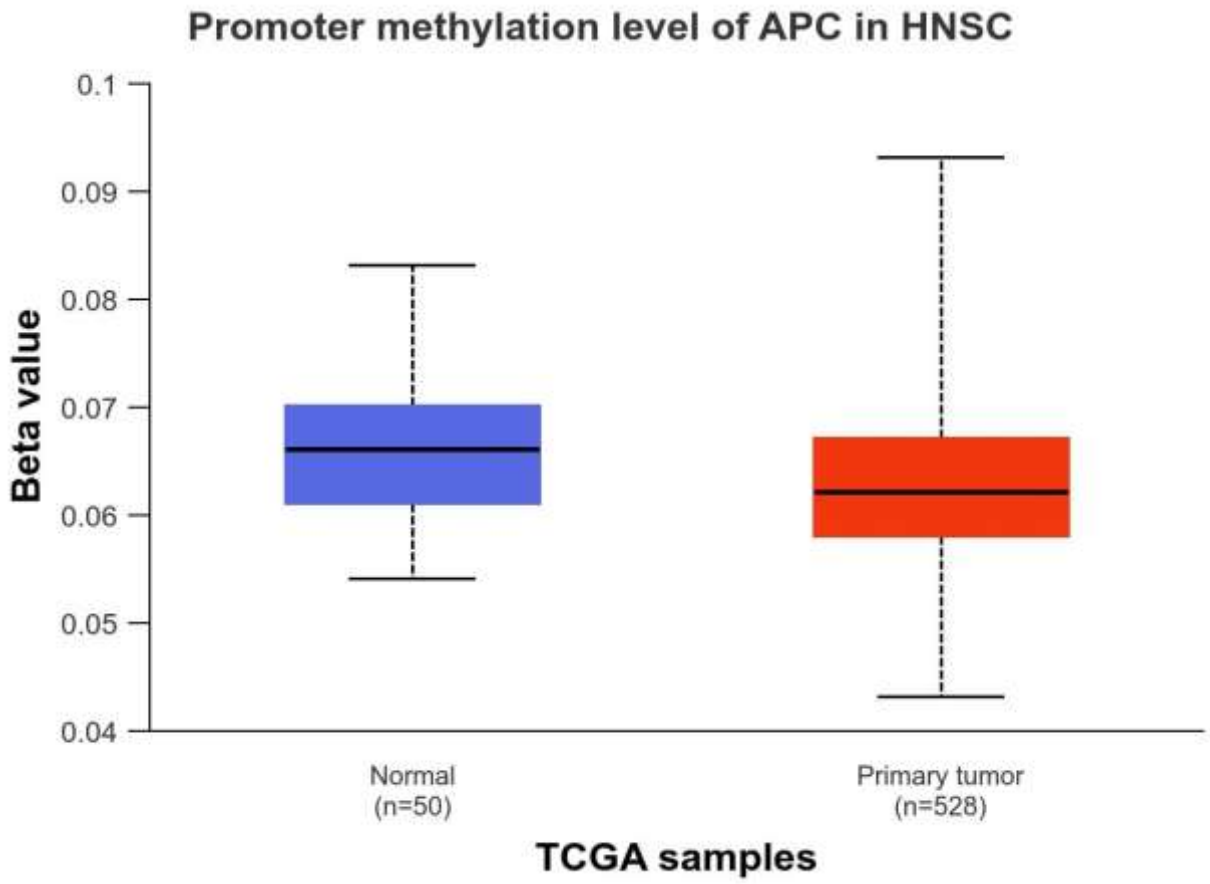

Figure 3: Promoter methylation of APC gene in HNSC and normal samples via the UALCAN. This figure shows the APC hypomethylation in HNSC patients as compare to normal sample by using UALCAN.

3.4 APC promoter methylation in HNSC sample according to various metrics

The age, gender, race, and cancer stage of the patients were all taken into account while assessing the promoter methylation of APC in HNSC patients. First, we discovered changes in APC promoter methylation at different stages of the HNSC. We detected significant hypomethylation at all stages of the HNSC, despite the fact that stages 1 and 4 had the same level of methylation as the control sample (Figure 4A). Next, we examined APC promoter methylation in different race groups of HNSC patients. When compared to normal samples, we found that Asian HNSC samples had significantly lower levels of APC expression. In contrast, Caucasian and African-American patients showed higher levels of hypomethylation than the Asian patient group, as seen in Figure 4B. The individual's gender-dependent APC promoter methylation levels in HNSC were then investigated. When compared to the control group, we found that the APC exhibited hypomethylation. The results also showed that the rate of hypomethylation in females was the same as in males, as shown in Figure 4C.

We then looked at the age-related variation in APC promoter methylation levels in patient samples: patients aged 61 to 80 and 81 to 100 had the same rate of hypomethylation, whereas patients aged 21 to 40 had a high degree of hypomethylation (Figure 4D). As a result, our data show that promotor methylation may be responsible for APC expression fluctuations.

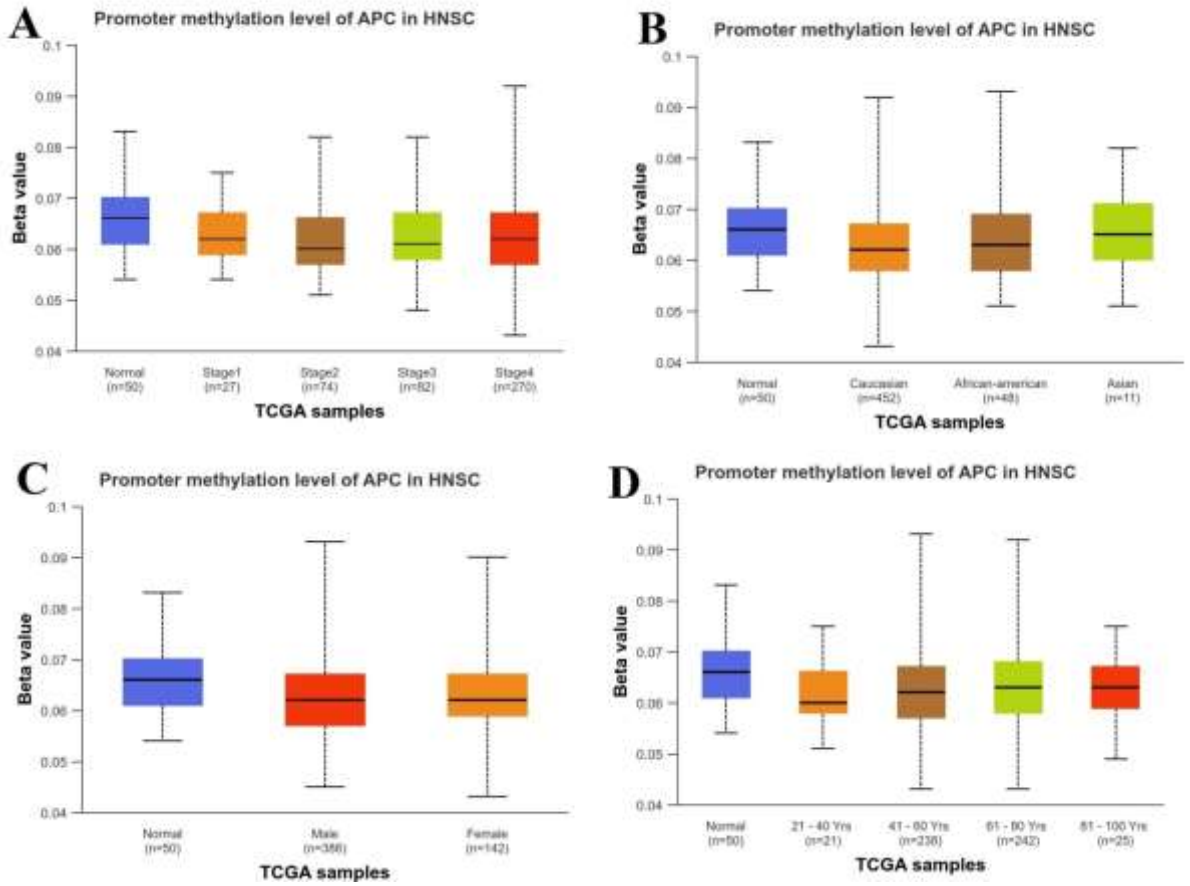

Figure 4: APC gene promoter methylation on the basis of different parameters. (A) Assessment of APC promoter methylation level in HNSC stratified according to disease phases. (B) Assessment of APC promoter methylation level in HNSC stratified according to ethinicity of patients. (C) Assessment of APC promoter methylation level in HNSC stratified according togender of patients. (D) Assessment of APC promoter methylation level in HNSC stratified according to age of patients.

3.5 Survival analysis of APC in HNSC patients

We evaluated patient OS using the KM plotter tool to determine the clinical importance of the APC gene in HNSC. As Figure 5 illustrates, we found that APC reduced expression has the impact on poorer survival of HNSC patients. APC downregulation results in the patient's lowest overall survival rate.

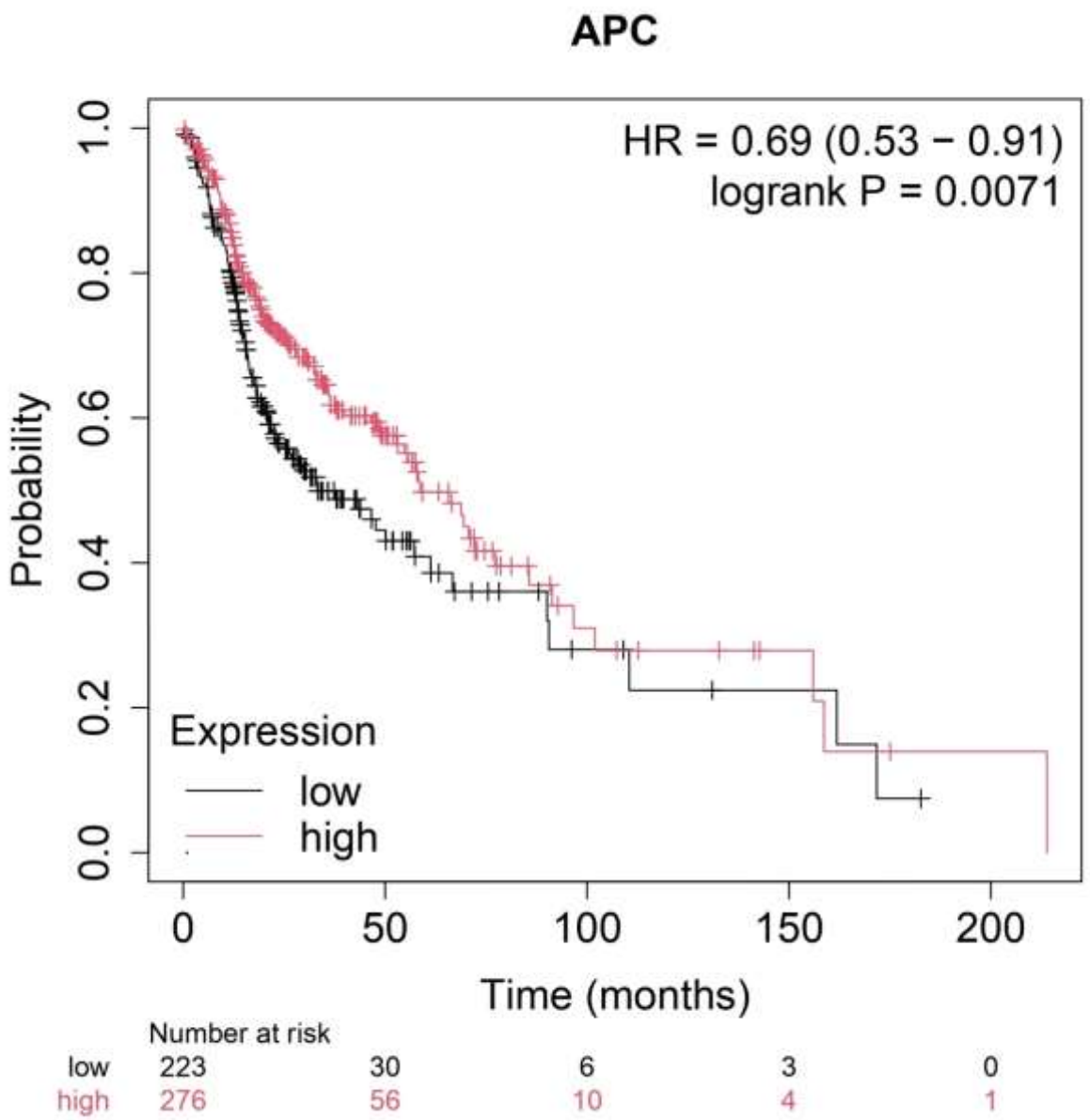

Figure 5: Survival analysis of APC gene in HNSC patients. This Figure shows that APC reduced expression has the impact on poorer survival of HNSC patients.

3.6 Validation of expression and survival analysis of APC in HNSC

We also used GEPIA2 to investigate APC expression and prognostic importance in parallel, hoping to validate our earlier results. Using the GEPIA2, we evaluated the expression of the APC gene in HNSC and control samples. The analysis revealed that HNSC samples expressed APC at lower levels than normal samples, although as Figure 6A illustrates, the results are not statistically significant. Next, we used the GEPIA2 stage plot module to assess APC expression in relation to the HNSC disease stages. Plotting the distribution of an APC expression over several cancer stages revealed that, as Figure 6B illustrates, the variable's values may differ between stages. However, these differences don't seem to be significant, as indicated by the estimated p-value of 0.626.

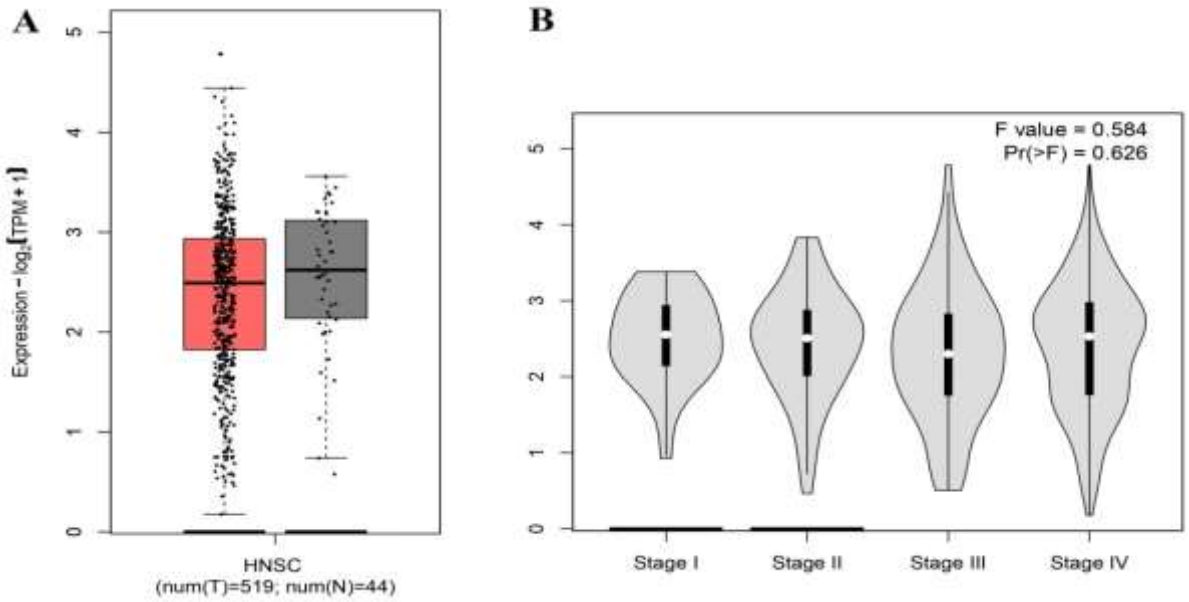

Figure 6: (A) GEPIA2 analysis of APC expression in HNSC. (B) Using GEPIA2, an analysis of APC expression in HNSC according to clinicopathological stage.

The results of survival analysis were verified by employing GEPIA2. Our results reveled that reduced expression associated poor overall survival (Figure 7). Nonetheless, the computed p-value of 0.15 revealed that the difference was not statistically significant, since the P-value was more than 0.05, and the estimated HR of 0.82 indicated an increased risk with high APC expression.

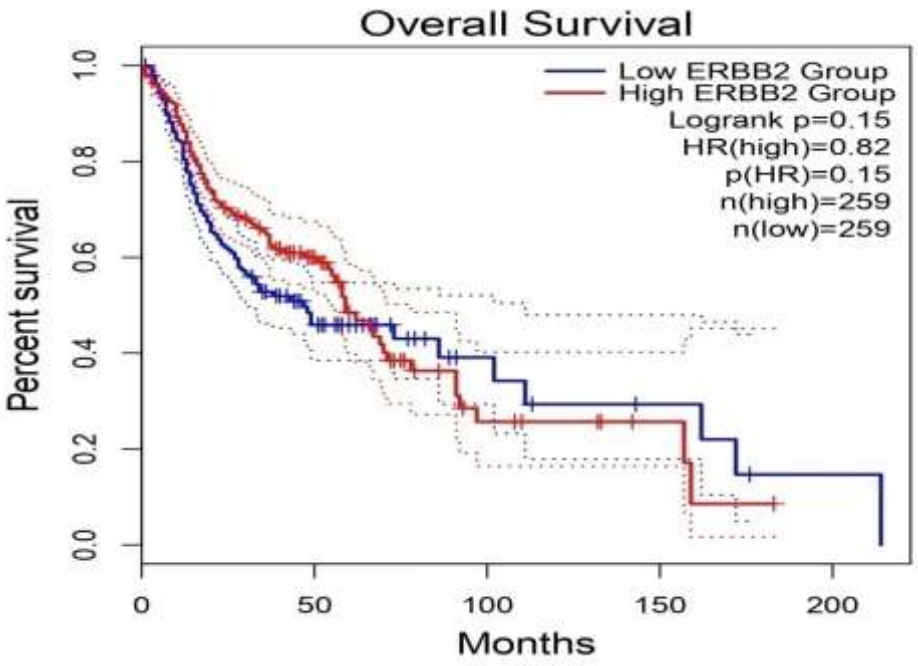

Figure 7: Survival analysis of APC gene in HNSC by using GEPIA2. This Figure demonstrated that low expression of APC gene in HNSC resulted in the lowest overall survival.

3.8 APC mutational analysis in HNSC

Using the cBioPortal platform, APC mutational analysis was performed on HNSC samples. In all analysed HNSC samples, we found a five percent mutation rate in the APC gene (Figure 8). As

illustrated in Figure 8, this gene was found to have three deep deletions, five truncating mutations (possible driver), and seventeen missense mutations (unknown relevance).

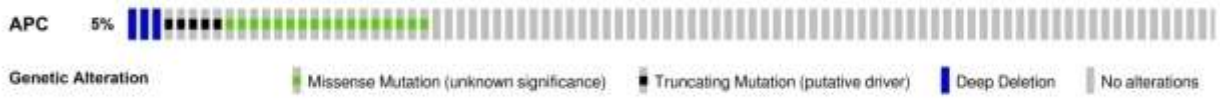

Figure 8: Analysis of APC gene mutations in patients with HNSC via cBioPortal

3.9 Correlation between APC genotype and other HNSC mutant genes.

Utilizing mu-Target, the APC gene correlation in HNSC was investigated in relation to other mutated genes. The top 5 genes that have been chosen and are associated with APC are GCNT4, AFG3L2, CCDC112, PRAGD and ADTRP. The APC gene expression has a negative correlation with GCNT4 and ADTR but a positive correlation with AFG3L2, CCDC112, and PRAGD, according to mu Target data (figure 9). All of these data together showed that APC and several other mutant genes in HNSC have high correlations.

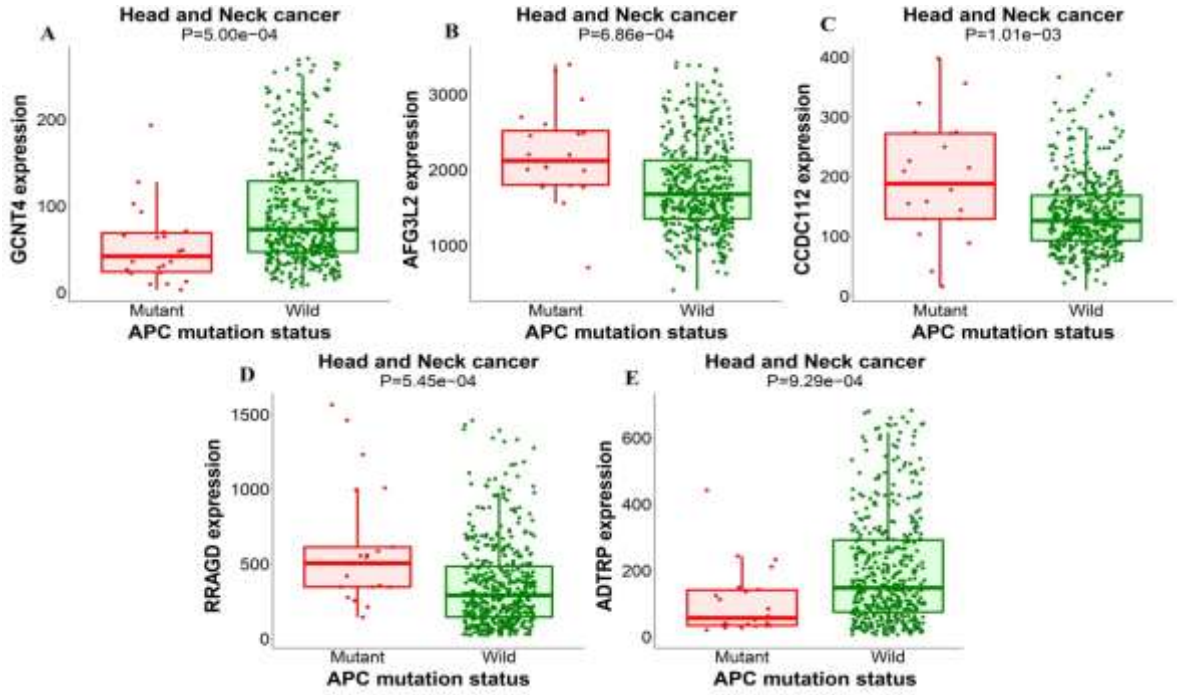

Figure 9: Correlation between mutated APC genes with other genes in HNSC cases. A) Negative correlation of APC gene with GCNT4, B) Positive correlation of APC gene with AFG3L2, C) Positive correlation of APC gene with CCDC112, D) Positive correlation of APC gene with PRAGD, E) Negative correlation of APC gene with ADTRP.

4 Discussions

The APC gene, which is clinically relevant in HNSC, was studied for expression, promoter methylation, survival evaluation their ratification, mutational analysis and correlation of APC gene with other mutated genes. We have produced several significant discoveries as a result of these investigations, and we evaluate the quality of our findings by contrasting and comparing them with those of earlier research.

Using the UALCAN database, we first examined APC gene expression in the HNSC and control samples. It was discovered that APC gene expression was down-regulated in HNSC samples. As seen in Figure 1, the P value of 7.4E-1 indicated that the results were not significant.

According to reports, APC gene expression was gradually downregulated up to stage 3, but by stage 4, the degree of downregulation was minimal. As can be seen in Figure 2A, the p values for stages 1, 2, 3, and 4 are statistically not significant because there P values are greater than 0.05. The TNM classification serves as a source for therapy indications as well as prognostic markers for cancer. The eighth version of the American Joint Committee on Cancer's (AJCC) staging classification for neoplasms was updated in 2016 [37]. Additionally, in 2017, the Union for International Cancer Control (UICC) released an updated TNM categorization of malignant tumors [38].

In a series of ACS reports published in the late 1980s, significant disparities in the cancer burden by race and ethnicity were documented [39]. All ethinic groups had downregulation, although Asians had a high degree of downregulation of APC, and the P value was statistically not significant across the board when compared to the control, as Figure 2B illustrates. Key ideas in many of the theoretical frameworks used in the development of cancer screening programs are concern and risk perceptions. Because most cancers for which we have early detection or prevention approaches are gender specific, few research have examined gender differences [40].

Gender-specific analysis of the APC gene expression revealed down-regulated target gene expression, as Figure 2C illustrates, the P value was statistically not significant when compared to the control group.

As illustrated in Figure 2D, when we examined the target gene expression according to age, we found that the age group 81–100 years old had the lowest level of downregulation, and their P value was not significant. Age is a risk factor for cancer that has been studied extensively and is expressed in completed units of time. It is employed in nearly all cancer epidemiological studies [41, 42].

We compared the APC gene promoter methylation in HNSC patients to normal individuals and found promoter hypomethylation of the APC gene with a significant P value of 1.6358E-12. DNA methylation and chromatin-modifying factors are examples of epigenetic alterations that regulate tumor-suppressor gene silence, proto-oncogene activation, and chromosomal instability, all of which are important aspects of the carcinogenesis process [43]. Promoter methylation is a crucial epigenetic alteration that significantly affects gene expression [44, 45].

Based on the cancer stage, we investigated the promoter methylation of the APC gene and noticed that promoter hypomethylation had occurred, with a high degree of promoter hypomethylation at stage 2 (Figure 4A). This was followed by an analysis of the APC gene promoter methylation in HNSC patients based on ethinic group. The results showed that the APC gene had promoter hypomethylation compared to normal and noticed that the degree of hypomethylation was higher in Caucasian patients compared to Asian and African-American patients. The differences between the Caucasian and African-American patients and the control group were significant, but not the Asian patients compared to the normal group (Figure 4B).

Following that, the APC gene promoter methylation was examined for each gender, and it was noticed that the promoter region in both males and females was equally hypomethylated. The results were noteworthy as the P value was less than 0.05 (Figure 4C).

Finally, we examined the target gene's promoter methylation across the various age groups and discovered that, as shown in Figure 4D. The first age group showed non-significant hypomethylation, while the latter two age groups—61–80 and 81–100 years old—showed equal hypomethylation.

While doing survival analysis, we also examined the relationship between the APC gene expression and survival analysis in patients with HNSC, and we discovered that the patient would have the lowest overall survival if the target gene was hyporegulated (Figure 5). A subgroup study revealed better survival for all areas of the body excepting the larynx, where survival was stationary, and all age categories except older patients, >75 years [46] According to earlier research, gene overexpression is linked to the development of cancer [47, 48]. Therefore, our findings pointed out an involvement of APC in the onset and development of HNSC. Subsequently research revealed that a reduced expression of APC was linked to a worst survival for HNSC patients. The results of

APC gene expression were also verified by using GEPIA2 (figure 6, 7). This study suggests that a decrease in APC expression causes HNSC to progress..

Additionally, cBioPortal was used for mutational analysis. As illustrated in Figure 8, we discovered that the APC gene in the HNSC sample had a mutation rate of 5%. Using populations based approach, we have calculated the lifetime cancer risk associated with germline DNA mismatch repair gene mutations, regardless of an individual's family history [49]. It is well recognized that chromosomal abnormalities, including translocations and the resulting gene fusions, duplications, deletions, and induced gene gains or losses, are crucial in the development of tumors [50]. The cBioPortal for Cancer Genomics is an online resource for analyzing, visualizing, and assessing multimodal cancer genomics data (http://cbioportal.org) [51]. Even though our findings indicated that genetic differences in HNSC patients had a negligible impact on APC gene dysregulation, more research is still required.

Next, It was identified that mutant APC gene expression was correlated with different mutant genes which was analysed by using mu Target tool. The top 5 mutant genes that were selected in HNSC were CCDC112, AFG3L2, GCNT4, and PRAGD. The APC gene expression had a negative correlation with GCNT4 and ADTR but a positive correlation with AFG3L2, CCDC112, and PRAGD, according to mu Target data all the results were significant because their P values were less than 0.05 (Figure 9).

5 Conclusions

In order to provide a thorough understanding of APC in patients with HNSC, our study focuses on promoter methylation status, expression models, predictive indicators, correlation of APC gene with other genes and mutation analysis. In summary, APC may be regarded as a unique prognostic biomarker for HNSC, and more research into its functioning mechanisms may make it possible to target APC for HNSC treatment.

6 Conflict of interest

The writers say they don't have any conflicting interests.

7 Acknowledgement

None

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