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Scientific Letters

*Review*

# Exploring the Clinical Relevance of p31$^{comet}$ in Head and Neck Squamous Cell Carcinoma through UALCAN database analysis

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**Abstract:** The p31$^{comet}$ protein plays a pivotal role in regulating spindle assembly checkpoint silencing and is overexpressed in several cancers, including oral cancer. Despite this, its exact roles in tumorigenesis and its prognostic significance remain unclear. In this study, using the UALCAN cancer database, we analyzed p31$^{comet}$ expression and its correlation with clinical indicators in Head and Neck Squamous Cell Carcinoma (HNSCC). Our findings revealed a significant upregulation of p31$^{comet}$ in HNSCC patients. Interestingly, we observed a positive correlation between p31$^{comet}$ expression and known interactors such as MAD2L1 and TRIP13, as well as regulators of p31$^{comet}$ expression. Furthermore, we found that p31$^{comet}$ expression was notably increased in tumor samples exhibiting alterations in the mTOR pathway and the SWI/SNF chromatin remodeling complexes. Intriguingly, HNSCC patients with high p31$^{comet}$ expression showed a tendency towards better prognosis compared to those with low/medium expression levels. This tendency did not reach statistical significance, likely due to variations in patient cohort sizes within the database. In summary, our findings suggest that increased p31$^{comet}$ expression could be a potential marker for tumor occurrence and metastasis in HNSCC patients, opening avenues for further research to understand its prognostic significance.

**Keywords:** bioinformatic analysis; p31$^{comet}$; tumorigenesis; prognostic; biomarker; head and neck squamous cell carcinoma

Received: 28 May 2024; Accepted: 08 July 2024; Published: 23 July 2024

## Introduction

Head and neck squamous cell carcinomas (HNSCCs) are the most prevalent malignancies originating from the mucosal epithelium in the oral cavity, pharynx, and larynx within the head and neck region [1]. In 2018, HNSCC ranked as the sixth most common cancer globally, accounting for 890,000 new cases and 450,000 deaths [2]. The prevalence of HNSCC is on an upward trend, with a projected 30% increase by 2030. Additionally, over half of HNSCC patients experience recurrence and metastasis within a three-year timeframe [3].

The current treatment for HNSCC includes surgery, radiation therapy, chemotherapy, targeted therapy, and immunotherapy. These modalities may be used alone or in combination, depending on the specific characteristics of the cancer and the patient's overall health [2]. Among the drugs used in chemotherapy, some are microtubule-targeting agents (MTAs), with paclitaxel being the classic example. MTAs are also

known as antimitotic agents since they target the microtubules of the mitotic spindle, a critical structure that allows accurate chromosome segregation during mitosis. Consequently, and depending on the activity of the spindle assembly checkpoint (SAC) surveillance mechanism [4-6], treatment with MTAs induces mitotic arrest, followed by an expected cell death [7]. Unfortunately, cancer cells can evade cell death, exhibiting resistance to treatment, thereby underscoring the challenges associated with treatment resistance. Globally, there is an urgent need to determine molecular biomarkers for predicting premalignant HNSCC lesion progression, forecasting survival, uncovering potential intervention targets, and anticipating responses to current therapeutic agents. In this context, several SAC components have been explored as potential targets for cancer treatment [8-10].

SAC activity relies on the assembly of the mitotic checkpoint complex (MCC) resulting from the association of key mitotic proteins mitotic arrest deficiency 2 (Mad2), budding uninhibited by benomyl 3 (Bub3), Bub1-related 1 (BubR1), and cell-division cycle protein 20 (Cdc20). At unattached kinetochores, the closed-Mad2 (C-Mad2) bound to Mad1 recruits open-Mad2 (O-Mad2), inducing its conformational change into C-Mad2, responsible for sequestering Cdc20 [11,12]. The Cdc20-C-Mad2 complex, in association with BubR1-Bub3, maintains the anaphase promoting complex/cyclosome (APC/C) inactive. This prevents the degradation of mitotic substrates—securin and cyclin B—by the 26S proteasome, thereby halting the initiation of anaphase and the exit from mitosis [4-6]. Once all chromosomes are properly attached and aligned at the metaphase plate, the SAC signaling must be turned off to promote the transition from metaphase to anaphase, and mitosis exit.

p31$^{comet}$, also known as Mitotic Arrest-Deficient 2-Like 1 Binding Protein (MAD2L1BP), is a key component of the SAC pathway [13,14]. For instance, depletion of p31$^{comet}$ prevents SAC silencing, while its overexpression overrides the SAC. Both can consequently lead to cell death [13,15]. Under normal physiological conditions, p31$^{comet}$ plays an important role in SAC silencing, ensuring the timely mitotic exit of cells. On the one hand, p31$^{comet}$ prevents Mad2 activation, by interacting with Mad1-bound C-Mad2, thus inhibiting the conversion of open O-Mad2 into C-Mad2 [16,17]. On the other hand, p31$^{comet}$ can promote MCC disassembly, by promoting Cdc20-bound C-Mad2 dissociation from BubR1, promoting Cdc20 autoubiquitylation and acting in concert with the ATPase TRIP13 to dissociate the Cdc20-C-Mad2 subcomplex and convert C-Mad2 back into its inactive form (O-Mad2) [18-23]. While this checkpoint component is vital for normal cell proliferation, its dysregulation can be catastrophic in the cancer context. In a previously reported study, we gathered information regarding p31$^{comet}$ mRNA expression in human cancer and found that p31$^{comet}$ is overexpressed, at mRNA levels, in several solid cancer types, including lung, breast, skin, parathyroid glands, bladder, brain, kidney, prostate, uterus, pancreas, and head and neck, as well as in leukemia [14].

The Cancer Genome Atlas (TCGA) project has yielded an extensive repository of genomic data, unveiling comprehensive molecular insights into a wide array of cancer types. TCGA's vast sample collection presents an invaluable resource for tackling challenges associated with the diversity within cancer. The TCGA support team has crafted a variety of computational tools tailored to facilitate precise data analysis. Among these resources, UALCAN emerges as a user-friendly, interactive web portal, empowering users to delve deep into their investigations of TCGA gene expression data [24]. A previous analysis of TCGA data showed that p31$^{comet}$ upregulation is associated with a poor prognosis in *BRCA1*-deficient breast cancer patients [25]. However, the relationship between this SAC component and disease progression in HNSCC patients is still poorly elucidated.

Exploring the complex interplay between p31$^{comet}$ and the clinical characteristics of HNSCC patients, as well as the potential impact of p31$^{comet}$ on HNSCC aggressiveness, presents an interesting avenue for predicting novel biomarkers in HNSCC and identifying innovative treatment targets. Here, we collected and analyzed common datasets in the UALCAN database to perform a comprehensive analysis of the expression and clinical significance of the p31$^{comet}$ gene in head and neck cancer.

The UALCAN web resource (http://ualcan.path.uab.edu/) was used for the analysis of p31$^{comet}$ expression in HNSCC, and its association with clinicopathological characteristics of HNSCC patients [26]. Accordingly, the screening conditions for filtering and conducting data were defined as follows: "Gene: MAD2L1BP" and "Cancer Type: Head and Neck Squamous Cell Carcinoma". The "Analysis Type" was defined according to the target variable, such as "HNSCC vs Normal Analysis". Within UALCAN, transcriptomic data were retrieved from TCGA, while proteomic data were obtained from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) [26,27]. For transcriptomic data, the results were provided by UALCAN as transcripts per million, while in proteomic information, the results were expressed as Z-values, which represented standard deviations from the median across samples for HNSCC. For correlation analysis, UALCAN used Pearson correlation analysis and provided the Pearson correlation coefficient values. For survival plots, Kaplan-Meyer curves were generated to express HNSCC patient survival according to p31$^{comet}$ expression and clinicopathological features. Data are shown as the means ± standard deviation (SD). Statistical significance, represented as *p*-values, was provided by UALCAN.

## p31$^{comet}$ regulation

The regulatory mechanism of p31$^{comet}$ activity in humans has been elusive, even though p31$^{comet}$ has been reported to be a phosphoprotein [28]. Accordingly, in *Xenopus* egg extracts, p31$^{comet}$ was shown to be

phosphorylated by Inhibitor of nuclear factor k-B kinase-β (IKK-β), leading to the promotion of p31$^{comet}$ activity in SAC silencing [29]. Recently, in human cells, it has been reported that Polo-like kinase 1 (PLK1) seemingly phosphorylates p31$^{comet}$ in a direct way in the serine 102 residue. The PLK1-mediated phosphorylation of p31$^{comet}$ inhibits the concerted activity of p31$^{comet}$ and TRIP13 in the disassembly of the MCC [30]. Nonetheless, the mechanism by which the phosphorylation of p31$^{comet}$ inhibits this activity still needs to be investigated.

Furthermore, p31$^{comet}$ activity is also regulated through the interaction with other proteins. For instance, the interaction of p31$^{comet}$ with both RAS-like without CAAX 1 (RIT1), a RAS GTPase, and Dynein intermediate chain 2c (DNCI2c), a Dynein subunit, has also been shown to be essential for p31$^{comet}$ SAC silencing function [23,31].

## p31$^{comet}$ functions beyond the SAC

Besides its role in SAC signaling, p31$^{comet}$ has also been implicated in several crucial cellular processes that will be discussed below.

It has been reported that p31$^{comet}$ plays a role in cohesin degradation by promoting the disassociation of Shugoshin 2 (SGO2)-Mad2 complex. SGO2, a well-known meiotic cohesin protector, when bound to active Mad2, competes with securin for the association with separase. Similarly to securin, this interaction leads to separase inhibition. p31$^{comet}$, in association with TRIP13, promotes the disassembly of separase-SGO2-C-Mad2, freeing separase. Free separase can then degrade cohesin and promote cell cycle progression [32].

Furthermore, p31$^{comet}$ has been shown to be involved in meiosis in several different species. In rice (*Oryza sativa* L.), p31$^{comet}$ is associated with both meiotic double-strand break (DSB) and Synaptonemal complex (SC) formation in a process mediated by Central region component 1 (CRC1), where it promotes the interaction between p31$^{comet}$ and ZEP1, a protein part of the SC [33]. Recently, several biallelic MAD2L1BP variants were implicated in female infertility by causing arrest in oocyte metaphase I. These variants led to truncated versions of p31$^{comet}$ that lost the ability to bind to Mad2, inhibiting p31$^{comet}$ SAC silencing function. Injection of full-length MAD2L1BP cDNA overcame this arrest, opening the possibility for a new therapeutic option for female infertility [34]. Moreover, in *C. elegans*, orthologous of p31$^{comet}$ and TRIP13 interact to promote proofread meiotic homologous interactions [35].

In *Arabidopsis*, COMET, the p31$^{comet}$ homologous, assists in removing ASY1 from the meiotic chromosome axis. ASY1 is the homologous of HORMAD1/2 in mammals and plays a role in homologous chromosome synapsis. HORMA domain proteins, vital for crossover formation and DNA recombination accuracy, are also negatively regulated by TRIP13 [36].

p31$^{comet}$ is also involved in the regulation of homologous recombination (HR) by promoting DSB resection by regulation of TRIP13 and REV7 interaction. REV7 is a HORMA protein that forms a complex with Shieldin (SHLD1-3), which is known to contribute to the protection of DNA ends from degradation, repressing HR. p31$^{comet}$ acts as a scaffold leading to the release of SHLD3 from REV7 and consequently to DSB end resection [25,37]. REV7 also interacts directly with p53. Recently, it was shown that the REV7-p53 complex leads to the inhibition of DNA damage signaling mediated by ATM [38]. TRIP13-mediated disassembly of the REV7-p53 complex is promoted by p31$^{comet}$, which acts like a scaffold for TRIP13 in this process. Furthermore, a recent study reported that a truncated p31$^{comet}$ protein resultant of a biallelic MAD2L1BP mutation, R253*, showed impaired binding ability to TRIP13, Mad2, and REV7, hindering p31$^{comet}$ activity in SAC silencing and HR. This mutation also hindered p31$^{comet}$ ability to interact with p53 [39].

p31$^{comet}$ has also been implicated in metabolic homeostasis. The insulin receptor (IR) is typically internalized by endocytosis. This process is mediated by clathrin and regulated by Adaptor protein 2 (AP2), a protein that facilitates the interaction between clathrin and cargo. Since BubR1 was found to interact with AP2 and Mad2 with the IR, it is suggested that Mad2 is a mediator in the interaction between BubR1-AP and the IR [40-42]. However, p31$^{comet}$ can hinder the interaction between BubR1-AP and Mad2-IR, possibly by binding to C-Mad2 in the same region as BubR1, and potentially disrupting IR internalization [43]. In post-mitotic neurons, the Serotonin transporter (SERT), which is involved in the regulation of serotonergic neurotransmission and neuromodulation, was showed to interact with AP2, BubR1 and p31$^{comet}$ in a process suggested to be dependent on Mad2 mediation, since depletion of Mad2 reduced this interaction. It was also suggested that SERT forms a complex with these proteins, promoting SERT endocytosis [44,45]. Additionally, p31$^{comet}$ knockout in mice led to decreased reserves of glycogen and was fatal after birth, while depletion in the liver caused reduced IR levels in hepatocyte plasma membrane, insulin resistance, hyperglycemia and hyperinsulinemia [40]. Nonetheless, the mechanisms behind p31$^{comet}$ involvement in these processes need to be further investigated.

## Anticancer strategy through p31$^{comet}$ targeting

The SAC plays a critical role in ensuring accurate chromosome segregation, making it a recent target for new antimitotic approaches [4]. Consequently, due to its significance in SAC regulation and its altered expression in cancer cells, p31$^{comet}$ has emerged as a potential target for anticancer therapies. Two strategies are being considered: first, overexpression of p31$^{comet}$ to effectively silence the SAC; and second,

inhibition of p31$^{comet}$ to delay SAC silencing, ultimately leading to cancer cell death. Additionally, modulation of p31$^{comet}$-derived peptides could serve as an alternative method to directly deactivate the APC/C, thereby prolonging mitotic arrest [46].

Aberrant shutdown of the SAC often leads to serious errors in chromosome segregation, which can prompt cell death or senescence, a permanent status of cell cycle arrest [4,47] . Overexpression of p31$^{comet}$, aimed at deactivating the SAC, has shown to be cytotoxic to different cancer cell lines, causing senescence or apoptosis in a manner dependent on its interaction with Mad2 [47-49]. This p31$^{comet}$-induced senescence is associated with signs of mitotic catastrophe, like micronucleation, abnormal nuclei and chromosomes, and anaphase bridges [47,48]. Interestingly, senescence induced by p31$^{comet}$ overexpression is not always linked to the presence of the p53 protein, except for A549 cells, where it relies on another tumor-suppressing protein, called p21Waf1/Cip1.

Cellular senescence has been recognized as a natural defense against tumor development, suggesting that enhancing p31$^{comet}$ expression could serve as a potential strategy against cancer [47,50]. However, senescence may also contribute to tumor progression by triggering inflammation and altering the surrounding tissue, creating a favorable environment for tumor growth [51]. Overexpression of p31$^{comet}$ has been observed in various human cancers and is linked to senescence. Moreover, increased levels of p31$^{comet}$ can make cancer cells resistant to drugs that target cell division by disrupting the SAC.

While boosting p31$^{comet}$ expression seems promising for combating cancer, it is crucial to consider the potential role of senescence in tumor development, as well as the risk of developing resistance to anti-cancer drugs.

## Increasing sensitivity to chemotherapeutic drugs through p31$^{comet}$ depletion

Different treatment strategies have been explored in cancer cells using the depletion of p31$^{comet}$ in combination with chemotherapeutic drugs. The most explored combination has been with MTAs. The rationale behind this strategy is that p31$^{comet}$ depletion can enhance the mitotic arrest promoted by MTAs by further sustaining an active SAC, potentially preventing or delaying mitotic slippage and exacerbating cell death. In fact, this was observed in several cancer cell lines, where the combination led to increased mitotic arrest duration, which translated into increased cell death [52].

More recently, in lung cancer cell lines, we reported similar findings with a lower concentration of paclitaxel. We also explored the combination of p31$^{comet}$ depletion with navitoclax, an inhibitor of the B-cell lymphoma 2 (BCL-2) prosurvival family members BCL-2 and BCL-xl. These proteins inhibit apoptosis by preventing the release of cytochrome c from mitochondria, which would lead to the activation of caspases, the effectors of cell death. Increasing apoptotic signaling in cells arrested in mitosis has been suggested to prevent mitotic slippage and should lead to increased mitotic cell death. Interestingly, our results showed that this combination instead led to increased postmitotic cell death after a delayed mitotic arrest [53].

In oral cancer cells, we also explored if the depletion of p31$^{comet}$ could potentiate Cisplatin-induced cytotoxicity [54]. Since the promotion of mitotic arrest in esophageal squamous cell carcinoma cells treated with Cisplatin was shown to increase DNA damage and consequently cell death, a similar result was also expected [55]. However, this treatment approach led to increased cell death only in the cell line less responsive to treatment with Cisplatin alone. A possible explanation might be the different p31$^{comet}$ expression levels or the different ratio between p31$^{comet}$ expression and its interactors across the cell lines.

## p31$^{comet}$ expression analysis in head and neck squamous carcinoma using the UALCAN resource

Previous analysis of p31$^{comet}$ mRNA using data retrieved from the Oncomine database revealed overexpression across several cancers, including head and neck – floor of the mouth carcinoma (www.oncomine.com) [14]. Using data from TCGA, the UALCAN analysis of transcript levels of p31$^{comet}$ showed it is upregulated in HNSCC primary tumors (median value of transcripts per million 1.59 times higher in tumor samples). In addition, p31$^{comet}$ was also overexpressed at protein levels, according to data from the CPTAC (UALCAN) (Fig. 1).

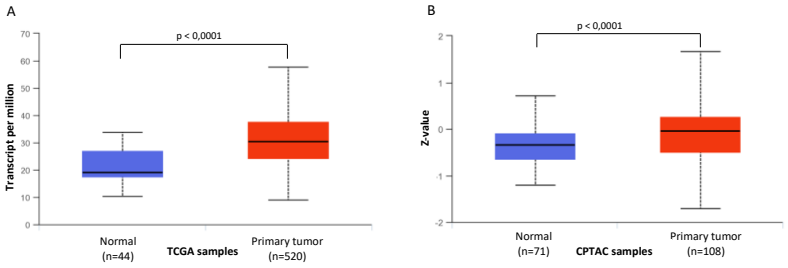



**Figure 1.** p31$^{comet}$ is overexpressed in head and neck squamous cell carcinoma (HNSCC) tissue. (A) mRNA expression of p31$^{comet}$ and (B) protein expression level. Boxplots were adapted from UALCAN. Statistical significance is represented as *p*-values.

## Relationship between p31$^{comet}$ expression in HNSCC and clinicopathological characteristics, assessed through UALCAN

Notably, p31$^{comet}$ showed a statistically significant overexpression or a tendency to be overexpressed across all tumor stages and grades relatively to normal samples, except for stage 2 at the protein level (Fig. 2 A-D). However, it is also worth noting that different cohorts were used for mRNA or protein analysis, as well as different sample sizes. For example, focusing on mRNA analysis (TCGA), 27 samples were evaluated from patients in stage 1, while the analysis of stage 4 comprised 264 samples. In the case of CPTAC samples (protein analysis), the *n* value was 71 in the normal cohort, and 7 in stage 1. On the other hand, considering stage 4, it was possible to observe a difference of 218 samples analyzed at mRNA ($n$ = 264) or protein ($n$ = 46) levels. Interestingly, p31$^{comet}$ upregulation in HNSCC was more accentuated in males at the mRNA level (median value of transcripts per million 1.63 times higher relative to normal samples, and 1.11 times higher when compared to female samples), a result also confirmed at the protein level (Fig. 2E, F).

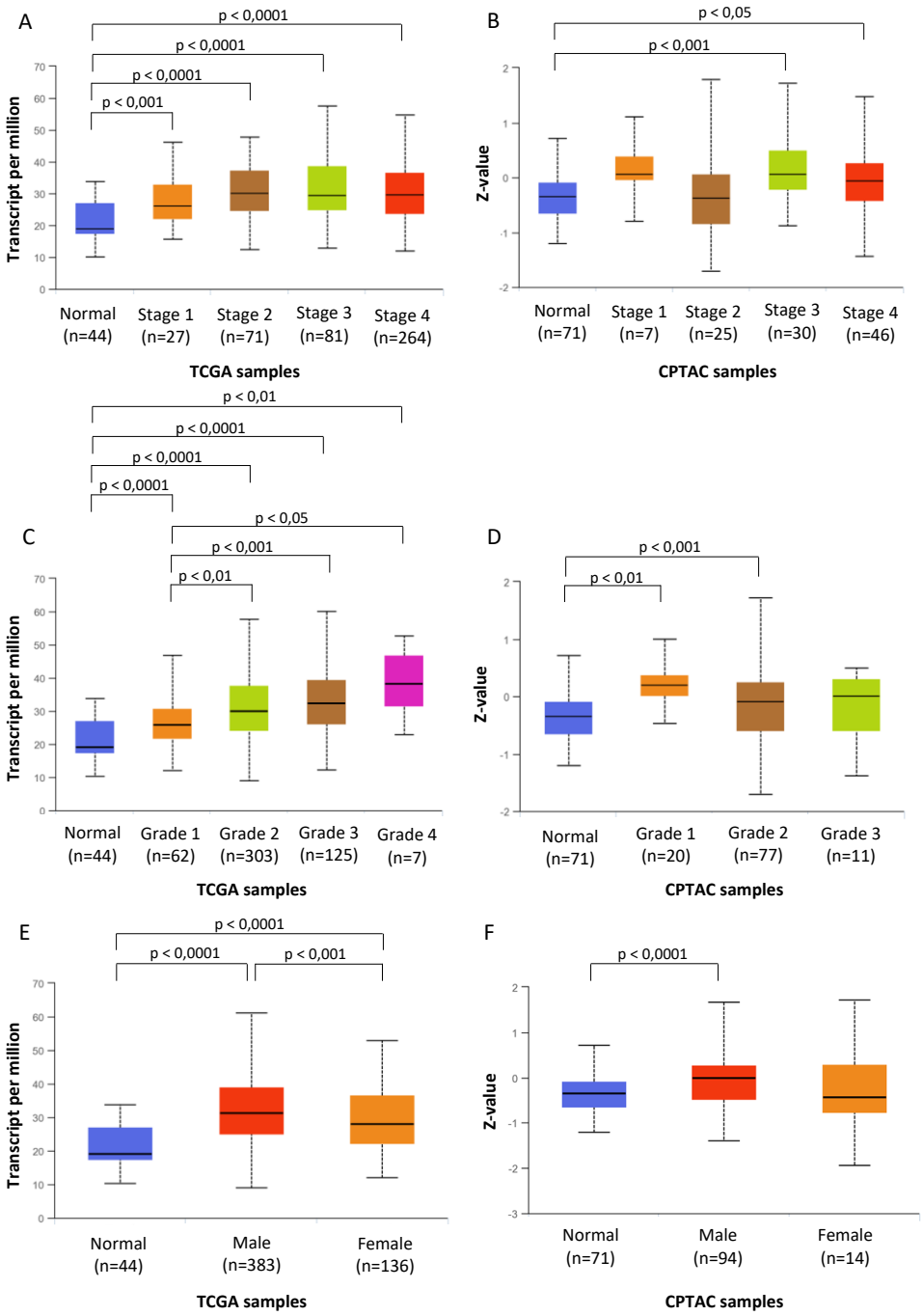



**Figure 2.** Relationship between p31$^{comet}$ expression in HNSCC and clinicopathological characteristics, assessed through UALCAN at mRNA (TCGA samples), and protein (CPTAC samples) levels. p31$^{comet}$ expression and tumor stage, tumor grade, and gender were analyzed at mRNA (A, C, and E) and protein (B, D, F) levels. Boxplots were adapted from UALCAN. Statistical significance provided by UALCAN is represented as *p*-values.

Remarkably, p31$^{comet}$ protein levels were higher in tumor samples featuring alterations in mammalian target of rapamycin (mTOR), HIPPO, nuclear factor erythroid 2-related factor 2 (NRF2), and receptor tyrosine kinases (RTK) pathways, as well as in the status of MYC/MYCN and SWItch/Sucrose Non-Fermentable (SWI/SNF) complexes (Fig. 3).

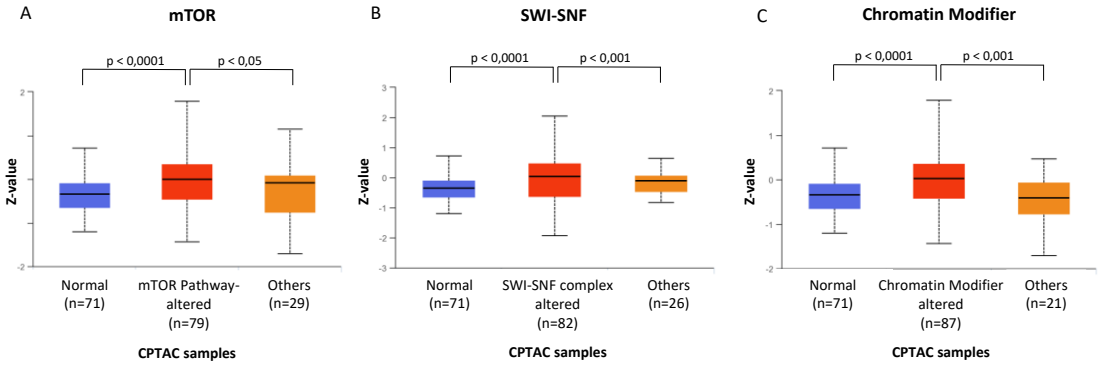



**Figure 3.** p31$^{comet}$ is upregulated in HNSCC cases (protein expression, CPTAC samples) with altered cancer-related pathways and regulators, as determined through UALCAN. (A) mTOR-altered pathway; (B) SWI-SNF-altered status; (C) chromatin-modifier status. Boxplots were adapted from UALCAN. Statistical significance provided by UALCAN is represented as *p*-values.

## p31comet is upregulated in HNSCC with altered cancer-related pathways

Dysregulation of mTOR, RTK, and/or NRF2 pathways, as well as altered MYC/MYCN and SWI/SNF status, are frequently associated with cancer [56-59]. Particularly, p31$^{comet}$ was significantly increased in tumor samples with changes in the mTOR pathway and SWI/SNF status (Fig. 3A, B). mTOR plays a key role in the modulation of metabolic pathways, being responsible for inducing several anabolic processes as a response to a wide range of environmental cues, and the mTOR pathway is frequently dysregulated in cancer [59]. SWI/SNF are ATP-dependent chromatin remodeling complexes with reported functions in DNA damage repair, and numerous cancers present mutations in the genes encoding for SWI/SNF subunits [60]. p31$^{comet}$ was also found to be upregulated in HNSCC cases where chromatin-modifier status was altered, compared with HNSCC samples that did not present this condition (Fig. 3C). This may be of interest since mutations in chromatin modifiers are associated with altered splicing profiles and contribute to carcinogenesis [61].

In addition to the aforementioned analysis of p31$^{comet}$ expression, a Pearson correlation analysis was also performed in UALCAN with mRNA transcript levels from TCGA, and Pearson correlation coefficient (Pearson-CC) values were provided. Among the genes whose expression was positively correlated with p31$^{comet}$ are MAD2L1 (Pearson-CC = 0.33), TRIP13 (Pearson-CC = 0.30), TP53 (Pearson-CC = 0.30), E2F3 (Pearson-CC = 0.38), E2F6 (Pearson-CC = 0.35), E2F7 (Pearson-CC = 0.36) and E2F8 (Pearson-CC = 0.30) (Fig. 4). In fact, as previously referred, it has been reported that senescence occurring in p53-proficient lung cancer cells after the induction of p31$^{comet}$ overexpression was dependent on p53 status and on p21 Waf1/Cip1 [47]. Furthermore, the mechanism of action of p31$^{comet}$ on SAC silencing is straightly connected to the interaction of p31$^{comet}$ with Mad2 and TRIP13 [14]. It must be noted that both p31$^{comet}$ and MAD2 expression are regulated by the Rb-E2F pathway [62]. Indeed, the coordinated expression of p31$^{comet}$ and MAD2 seems to be significant in cancer development and/or treatment, as the p31$^{comet}$/Mad2 ratio was reported to be altered in some cancers, and different p31$^{comet}$/Mad2 ratios were proposed to be associated with the type of response of cancer cells to spindle poisons (sensitive *versus* resistant) [52]. The observed patterns of p31$^{comet}$ expression point to it as an interesting candidate as therapeutic target in HNSCC. Patient survival as a function of p31$^{comet}$ expression was then assessed.

## Relationship between clinicopathological features and HNSCC patient survival

The survival probability of HNSCC patients over time, according to p31$^{comet}$ expression and other clinicopathological characteristics, was also assessed through UALCAN. A Kaplan-Meier analysis was performed, based on TCGA data. Unexpectedly, and although not statistically significant, HNSCC patients with high p31$^{comet}$ expression showed a tendency towards a better outcome than those with low/medium p31$^{comet}$ expression (Fig. 5A). Nonetheless, it is also important to note that the cohorts have different sizes (129 patients with high p31$^{comet}$ expression, and 390 with low/medium p31$^{comet}$ expression), a fact that might influence the results, as the group of HNSCC patients with high p31$^{comet}$ expression is smaller. Furthermore, it is possible to observe different scenarios, if other variables are considered, such as tumor grade and patient race and gender (Fig. 5B-D). For instance, a higher expression of p31$^{comet}$ in samples from patients with tumor grade 2 is linked to worse survival, when compared with samples from tumor 2-grade patients with low/medium p31$^{comet}$ expression, after 2,000 days. In addition, female samples with high p31$^{comet}$ expression had lower survival probability, compared to female samples with low/medium

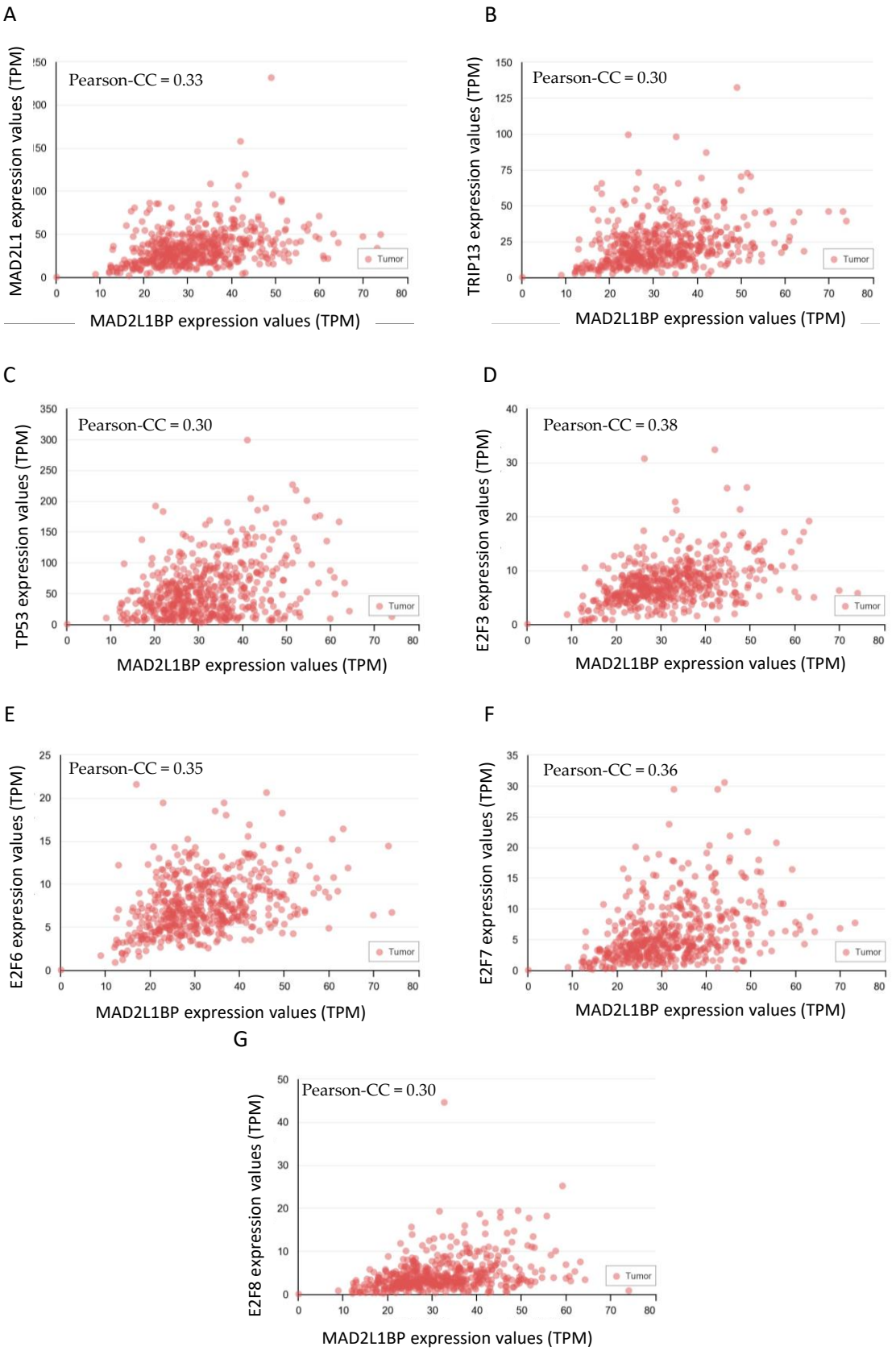
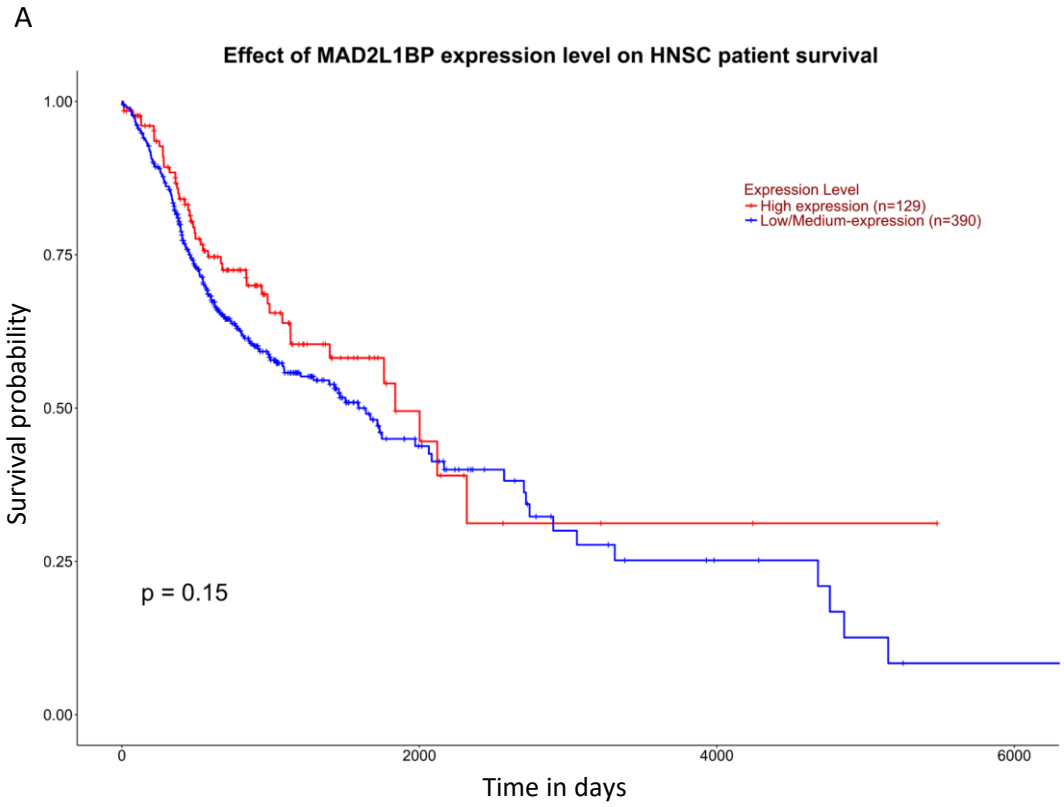
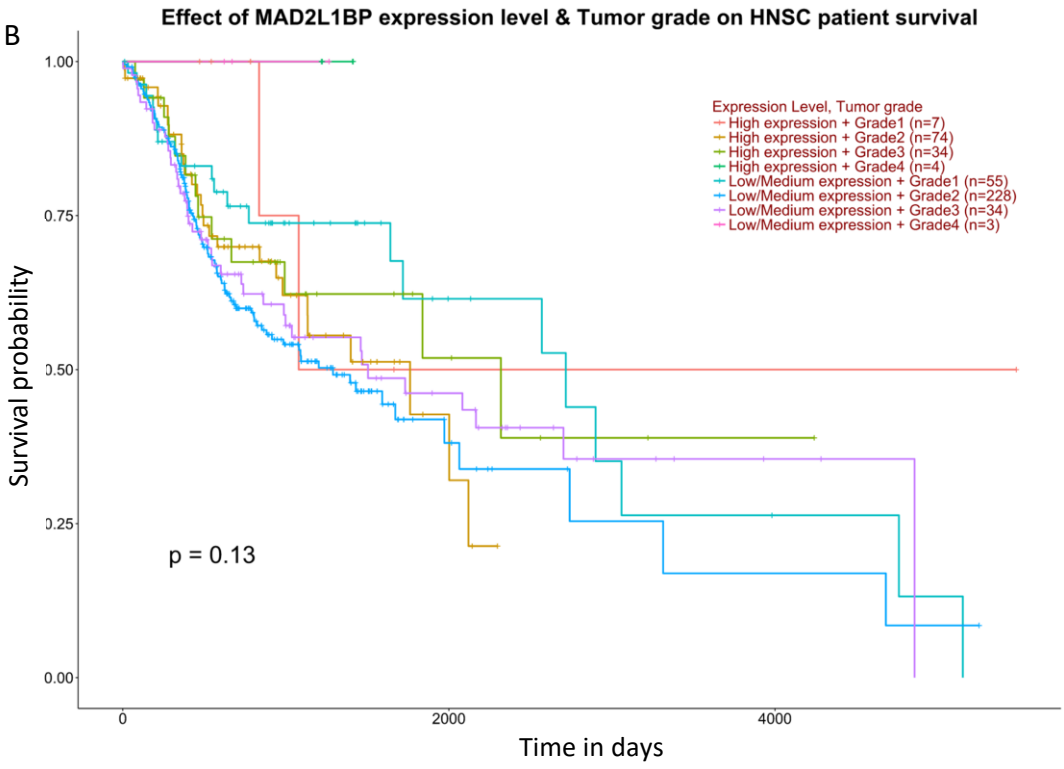



**Figure 4.** Pearson correlation analysis using gene expression between p31$^{comet}$ and MAD2 (A), TRIP13 (B), TP53 (C), E2F3 (D), E2F6 (E), E2F7 (F), and E2F8 (G) in HNSCC. Pearson correlation coefficient values, Pearson-CC, are shown for each analysis. TPM: transcripts per kilobase million.

p31$^{comet}$ expression, or with male samples in general. Regardless, this data does not allow to conclude about the significance of p31$^{comet}$ as a prognostic biomarker. However, it must be noted that p31$^{comet}$ acts in concert with other molecules, such as Mad2 and TRIP13 [14]. As such, it would not be surprising that a balance between these molecules could be more determinant in a clinical context than the individual expression of each of these partners. Therefore, the impact of p31$^{comet}$ expression in the survival of HNSCC patients should not be performed separately from these partners. These findings suggest that the possible role of p31$^{comet}$ as a prognostic marker in HNSCC should be analyzed in a more global context and include p31$^{comet}$ interactors.



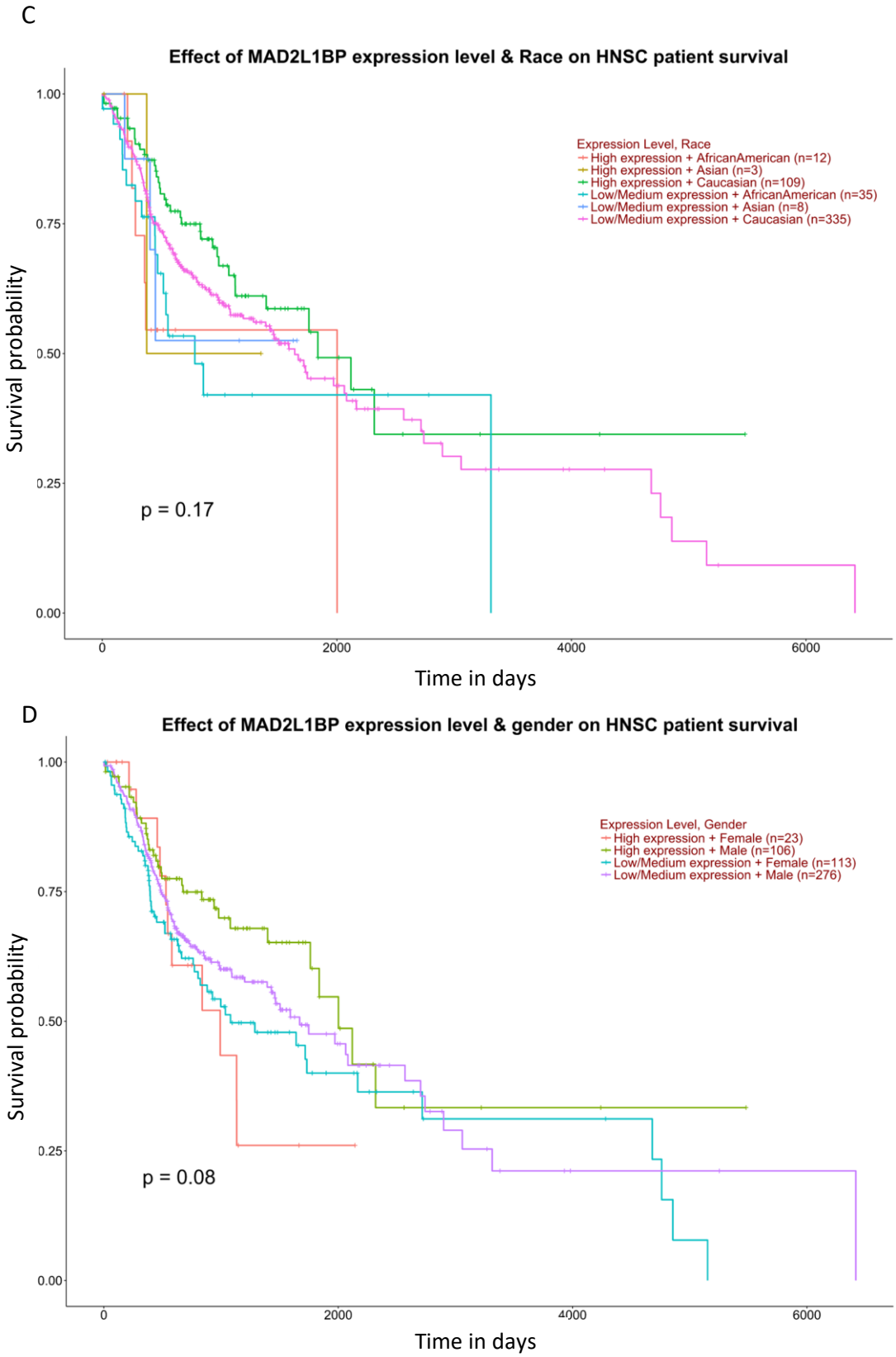



**Figure 5.** Kaplan-Meier curves for patient's survival according to p31$^{comet}$ expression and/or clinicopathological features. (A) p31$^{comet}$ expression; (B) p31$^{comet}$ expression and tumor grade; (C) p31$^{comet}$ expression and race; (D) p31$^{comet}$ expression and gender. *p*-values indicate statistical significance of survival correlation between groups. HNSC: head and neck squamous carcinoma; MAD2L1BP: mitotic arrest-deficient 2-like 1 binding protein.

## Discussion

We previously reported the potential of the SAC regulator p31$^{comet}$ as a target for cancer therapy [14,53]. In this study, we conducted a bioinformatic analysis to elucidate the relevance of p31$^{comet}$ in the pathogenesis and prognosis of HNSCC.

To investigate p31$^{comet}$ significance as a biomarker, the UALCAN web resource was used to assess transcriptomics and proteomics data on HNSCC samples and to analyze their association with clinicopathological features. The fact that p31$^{comet}$ was overexpressed across HNSCC samples at the mRNA and protein levels was in line with previous p31$^{comet}$ transcriptomics analysis based on Oncomine data [14]. Moreover, p31$^{comet}$ mRNA levels were upregulated in a wide range of cancers, according to data from Oncomine and TCGA. These results suggest a clinical relevance for p31$^{comet}$ in oral cancer, either as a prognostic biomarker, or as a therapeutic target. To reinforce this premise, it was also found that p31$^{comet}$ overexpression in HNSCC cases coincided with alterations in pathways or molecular regulators that are often associated with carcinogenesis, mainly in the mTOR pathway, and SWI/SNF chromatin remodeling complexes. Surprisingly, HNSCC samples with low or medium expression of p31$^{comet}$ showed a strong tendency towards a poorer survival than those where p31$^{comet}$ was upregulated. However, this result may also be influenced by other factors, such as gender and tumor grade. Curiously, preliminary results from our lab, based on tissue microarray analysis (TMAs) of oral cancer samples, also indicated that the cases with high p31$^{comet}$ levels were related to a better prognosis [unpublished data]. Nevertheless, it should be noted that p31$^{comet}$ function depends on the interaction with other partners, such as Mad2 and TRIP13, and that their expression should also be considered in survival analysis. Therefore, studying the changes occurring in the balance between those molecules can be more relevant than the analysis of their isolated expression. In agreement, altered p31$^{comet}$/Mad2 ratios were found in hepatocellular carcinoma and non-small cell lung cancer cells [52]. Moreover, the Pearson correlation analysis, performed through UALCAN, demonstrated that p31$^{comet}$ expression was positively correlated with the expression of its interactors MAD2 and TRIP13, as well as with genes that may be involved in the regulation of its expression, like E2F genes [62]. Notably, Mad2 mRNA levels were previously found to be upregulated in oral squamous cell carcinoma (OSCC) cells and immunohistochemistry analysis also revealed Mad2 overexpression in OSCC cases [63]. In addition, immunoexpression analysis demonstrated overexpression of Mad2 in oral leukoplakias [64]. Interestingly, it was also observed that a high expression of TRIP13 in HNSCC TMAs was associated with poor recurrence-free survival [65].

In conclusion, our analysis of p31$^{comet}$ expression in HNSCC revealed upregulation among HNSCC patients. Its positive correlation with known interactors and regulators suggests its involvement in critical pathways associated with cancer, such as the Rb-E2F and mTOR pathways. While elevated expression of p31$^{comet}$ may indicate its potential as a marker for tumor occurrence and metastasis in HNSCC, the inconclusive prognostic significance underscores the need for further investigations involving larger and more standardized patient cohorts, as well as an exploration of the relative expression of p31$^{comet}$ interactors. Importantly, conducting more in-depth studies to understand the specific molecular mechanisms by which p31$^{comet}$ influences oral carcinogenesis will provide valuable insights. The strategy could involve *in vitro* functional studies, including assays for cell proliferation, migration, and invasion, as well as the exploration of p31$^{comet}$ role in resistance to conventional treatments. These studies aim to examine both the impact of p31$^{comet}$ over- and underexpression and the signaling pathways involved in oral cell transformation. This approach could offer a more comprehensive understanding of this issue and potentially lead to significant advances in the prognosis, diagnosis, and/or treatment of oral cancer. Importantly, the present study highlighted the scarcity of information regarding the targeting of p31$^{comet}$ and underscored the need to explore its potential in other types of cancers beyond head and neck cancer, as it has never been evaluated as a prognostic factor.

## Acknowledgments

This research was funded by CESPU - Cooperativa de Ensino Superior Politécnico e Universitário Crl (Grants Ref. AntiMitoSphere_APSFCT_IINFACTS_2021, Ref. Flav4Tumor-GI2-CESPU-2022, Ref. SGA4Cancer-GI2-CESPU-2022, and Ref. upPTXovcar-GI2-CESPU-2022) to HB and PMAS. BP gratefully acknowledges CESPU (BD/CBAS/CESPU/01/2020) and Fundação para a Ciência e a Tecnologia (FCT) (2022.09451.BD) for financial support. JPNS gratefully acknowledges CESPU (BD/CBAS/CESPU/01/2021) for financial support. ACH gratefully acknowledges CESPU and Fundação para a Ciência e a Tecnologia (SFRH/BD/116167/2016).

## Author Contributions

ACH was involved in the work's conceptualization, methodology, formal analysis, investigation, data curation, original draft preparation, review, and editing. JPNS and BP contributed to formal analysis and to the draft review and editing. PMAS participated in formal analysis, draft review and editing, project administration and funding acquisition. HB was involved in the work's conceptualization, methodology, formal analysis, resources, draft review and editing, supervision, project administration and funding acquisition. All authors read and approved the final manuscript.

## Conflicts of interest

The authors declare no competing interests.

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