Overexpression of HPRT1 is associated with poor prognosis in head and neck squamous cell carcinoma

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Hypoxanthine phosphoribosyltransferase (HPRT1), as a salvage pathway enzyme, plays a crucial role in modulating the cell cycle and has been reported to be overexpressed in multiple cancers. Nevertheless, the relationship between the HPRT1 gene and head and neck squamous cell carcinomas (HNSCCs) has not been investigated so far. In this study, we first evaluated the expression and clinical value of HPRT1 mRNA and protein in tumor and healthy control tissues. Then, we examined mutations of the HPRT1 gene and their association with survival outcomes of patients with HNSCC. We also performed functional analyses of HPRT1 coexpressed genes and examined the association between HPRT1 expression and drug sensitivity. Both HPRT1 mRNA and protein were significantly higher in HNSCC compared with normal tissues, and up-regulation of HPRT1 was also correlated with age, sex, pathological stage and histological grades of patients with HNSCC. Moreover, HPRT1 and its associated genes were observed to be enriched for several cancer-related pathways, including DNA replication and cell cycle. Finally, patients exhibiting overexpression of the HPRT1 gene may be resistant to abiraterone and sensitive to several drugs, including tozasertib and teniposide. This study demonstrated that the elevated expression of HPRT1 gene is correlated with the progression of HNSCC; thus, this gene may serve as a useful indicator for the early detection, risk stratification and targeted therapy of patients with HNSCC.

Abbreviations
AUC, area under the ROC curve; BP, biological processes; CC, cellular component; CI, confidence interval; COSMIC, catalogue of somatic mutations in cancer; GEO, Gene Expression Omnibus; GO, Gene Ontology; HNSCC, head and neck squamous cell carcinoma; HPA, Human Protein Atlas; HPRT1, hypoxanthine phosphoribosyltransferase; HPV, human papillomavirus; IHC, immunohistochemistry; KEGG, Kyoto Encyclopedia of Genes and Genomes; LSCC, laryngeal squamous cell carcinoma; OS, overall survival; OSCC, oral squamous cell carcinoma; RFS, relapse-free survival; ROC, receiver operating characteristic; SDHA, succinate dehydrogenase complex flavoprotein subunit A; TCGA, The Cancer Genome Atlas; UCSC, University of California Santa Cruz.
Head and neck squamous cell carcinoma (HNSCC) has been introduced as the sixth most common malignancy globally. HNSCC originates from various subsites of the upper aerodigestive tract, such as the oral cavity, larynx, pharynx, paranasal sinuses and nasal cavity [1–4]. Despite current efforts for the risk stratification and treatment of patients with HNSCC, the overall survival (OS) rate of these patients is unsatisfactory and has not significantly improved over the past decade, which may be because of late-stage diagnosis. The lack of effective means for clinical applications urgently demands the findings of more applicable biomarkers to improve the earlier detection and targeted therapies of patients with HNSCC [5–11].

Hypoxanthine phosphoribosyltransferase (HPRT1) transcribes the HPRT protein, a transferase enzyme that takes part in the cell cycle through modulation of guanine and inosine production in the salvage pathway [12,13]. Although the HPRT1 is broadly used as a housekeeping gene for many expression studies, growing evidence has ascertained the differential expression of HPRT1 and its imperative role in quickly proliferating cells, such as neoplasms, due to elevated demand for nucleotides synthesis and consequently the HPRT1 during the cell cycle [14,15]. For instance, Townsend et al. [16] have shown the overexpression of HPRT1 in colorectal cancer samples compared with healthy tissues. Besides, others have demonstrated that the increased expression of HPRT1 in endometrial cancer samples was correlated with the survival outcomes of patients [17]. Moreover, Sedano et al. [18] also have discovered that up-regulation of the HPRT1 gene in tumor tissues could estimate the prognosis of patients with breast cancer. These findings provoked us to appraise the HPRT1 gene as a probable biomarker for patients with HNSCC.

In this study, we first analyzed the expression patterns of the HPRT1 gene in HNSCC using bioinformatics and laboratory investigations. We also assessed the diagnostic value and prognostic significance of the HPRT1 mRNA expression in patients with HNSCC. Besides, we conducted the mutation analysis for the HPRT1 gene in HNSCC. Finally, we explored those genes closely associated with HPRT1 in HNSCC and then performed functional enrichment analysis for these coexpressed genes to better understand the role of HPRT1 in HNSCC.

**Materials and methods**

**Expression analysis using the TCGA–HNSCC cohort**

The University of California Santa Cruz (UCSC) Xena browser (https://xenabrowser.net/) is an online web tool for analyzing and visualizing multiomic data and related clinical and phenotypic annotations [19]. We downloaded the mRNA expression levels of the HPRT1 gene and the information of several clinicopathological parameters of 520 HNSCC samples and 44 normal tissues in The Cancer Genome Atlas (TCGA) cohort from data deposited in the UCSC Xena browser. The clinicopathological features of patients of this cohort are listed in Table 1.

**Table 1. Clinicopathological features of patients in the TCGA–HNSCC cohort. NA, not available.**

| Clinicopathological parameter | Category | n   | Percent (%) |
|-------------------------------|----------|-----|-------------|
| Tissue specimens              | HNSCC    | 520 | 92.2        |
|                               | Noncancerous | 44  | 7.8         |
| Age (years)                   | 20–40    | 24  | 4.3         |
|                               | 41–60    | 249 | 44.1        |
|                               | 61–80    | 262 | 46.5        |
|                               | 81–100   | 27  | 4.8         |
|                               | NA       | 2   | 0.4         |
| Sex                           | Female   | 150 | 26.6        |
|                               | Male     | 414 | 73.4        |
| Stage                         | I        | 27  | 4.8         |
|                               | II       | 74  | 13.1        |
|                               | III      | 81  | 14.4        |
|                               | IV       | 266 | 47.2        |
|                               | NA       | 116 | 20.6        |
| Grade                         | G1       | 62  | 11.0        |
|                               | G2       | 304 | 53.9        |
|                               | G3       | 125 | 22.2        |
|                               | G4       | 7   | 1.2         |
|                               | NA       | 66  | 11.7        |

**Expression analysis using the Gene Expression Omnibus database**

The Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) is a valuable resource of available gene expression data that can be integrated and explored to derive new theories and knowledge [20]. We compared the HPRT1 mRNA expression in 23 HNSCC and 23 noncancerous tissues using a previously published dataset (GSE107591) [21] obtained from the GEO database. A P value <0.05 was considered statistically significant.

**Sample collection**

We obtained a total of 45 tumor tissues and paired adjacent normal tissues from 45 patients with HNSCC undergoing routine surgical procedures in the Amir Alam Hospital Complex in Tehran, Iran. It is noteworthy that none of the participants received treatment before the operation. The tissue samples were frozen immediately after surgery in liquid nitrogen and were stored at −80 °C until
RNA extraction. The demographic and clinical data of study participants are shown in Table 2. This study was carried out in accordance with the standards of the Declaration of Helsinki and with ethics approval from the Ethics Committee of Hormozgan University of Medical Sciences (IR.HUMS.REC.1399.150). Written informed consent was signed by all patients before involvement in this study.

RNA isolation and quantitative real-time PCR analysis

Total RNA from the patient’s tissue samples was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) based on the manufacturer’s protocol. According to the manufacturer’s protocol, the SuperScript IV Reverse Transcriptase cDNA Synthesis Kit (Roche, Germany) was used for cDNA synthesis. LightCycler 96 Real-Time PCR was used for the assessment of relative mRNA expression of the HPRT1 gene using SYBR Premix Ex Taq II (Tli RNaseH Plus; TAKARA, Japan) under the manufacturer’s protocol. Each experiment was conducted in duplicate, and the relative expression level was calculated using the –dct method normalized with Succinate Dehydrogenase Complex Flavoprotein Subunit A (SDHA). The primer sequences used in our study are deposited in Table 3.

Immunohistochemistry staining

The Human Protein Atlas (HPA) database (https://www.proteinatlas.org/pathology) is the largest and most comprehensive repository for proteins in tissues and cells, supplying vital data for assessment of expression profile at a single-cell resolution [22–24]. We evaluated the expression of the HPRT1 gene at protein levels in four HNSCC tissues and one oral mucosa using the immunohistochemistry (IHC) staining data (antibody CAB012200) collected from the HPA database.

Table 2. Clinicopathological features of patients in the validation cohort.

| Clinicopathological parameter | Category | n  | Percent (%) |
|-------------------------------|----------|----|-------------|
| Tissue specimens              | HN5CC    | 45 | 50.0        |
|                               | Noncancerous | 45 | 50.0        |
| Age (years)                   | <60      | 30 | 66.7        |
|                               | ≥60      | 15 | 33.3        |
| Sex                           | Female   | 33 | 73.3        |
|                               | Male     | 12 | 26.7        |
| Stage                         | I–II     | 18 | 40.0        |
|                               | III–IV   | 27 | 60.0        |
| Grade                         | G1       | 9  | 20.0        |
|                               | G2       | 15 | 33.3        |
|                               | G3       | 21 | 46.7        |

Table 3. Primer sequences used in this study.

| Quantitative real-time PCR primer | Type of primer | Sequence                   |
|-----------------------------------|----------------|----------------------------|
| HPRT1 (target gene)               | Forward primer | 5’-TGGCGTCCGTGATTAGTGATG-3’ |
| HPRT1 (target gene)               | Reverse primer | 5’-ACAGAGGGCTACAATGTGATG-3’ |
| SDHA (reference gene)             | Forward primer | 5’-CTTGCAGGACCTAGTAGTTGT-3’ |
| SDHA (reference gene)             | Reverse primer | 5’-CTCTCCACGACATCCTTCGG-3’ |

Prognosis analysis

The Kaplan–Meier plotter (http://kmplot.com/analysis) is an open-access resource that can be exploited to estimate the impact of 54,675 genes on survival outcomes of patients in 21 cancer types [25,26]. We obtained the information about the prognostic value of the HPRT1 mRNA expression in HNSCC through this database. A log rank P value <0.05 was considered statistically significant.

Mutation analysis

The Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://cancer.sanger.ac.uk) is a website for studying the influence of somatic mutations in all types of human cancers [27]. We introduced this database to figure out the distribution and substitutions of the HPRT1 gene mutations in HNSCC. The data have been imported to Excel 2019 software to illustrate the pie chart. Besides, we investigated the genetic alterations of the HPRT1 gene and their impact on the survival outcomes of patients with HNSCC using the data from the cBio Cancer Genomics Portal (http://cbioportal.org) database, a freely available platform for cancer genomic datasets [28–30]. A log rank P value <0.05 was considered statistically significant.

Coexpression analysis

We investigated the coexpressed genes of the HPRT1 gene in HNSCC through the UALCAN database. Genes with extremely low expression (median transcripts per million < 0.5) were removed, and only genes with a [Pearson’s correlation coefficient] ≥ 0.3 were involved. We then evaluated the expression profile of the collected genes in the GEPIA2 database (http://gepia2.cancer-pku.cn/#index), and only those genes that differentially expressed (Q < 0.01, [log2 fold change] > 1) were included for further analyses.

Functional enrichment analysis

We applied the Enrichr database (http://amp.pharm.mssm.edu/Enrichr) to carry out the Gene Ontology (GO)
and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis for coexpressed genes of the HPRT1 gene that differentially expressed in HNSCC. The Enrichr database is an extensive platform for curated gene sets and a search engine that gives biological awareness for further biological hypothesis [31]. The GO analysis contained biological processes (BP), molecular function and cellular component (CC). The retrieved terms were imported to Microsoft Excel 2019, and after selecting those terms with an adjusted $P < 0.01$, they were ranked based on the combined score. This index was produced by the Enrichr database and multiplied the log (Fisher’s exact test $P$ value) by z-score deviation from the expected rank. The bar chart for the top 10 terms in each category was illustrated using Microsoft Excel 2019.

Drug sensitivity analysis

To demonstrate whether aberrant expression of the HPRT1 gene could influence the clinical response to treatment and could be considered a potential biomarker for drug screening, we performed drug sensitivity analysis through the GSCALite database. This database has accumulated 481 and 265 small molecules from the Therapeutics Response Portal and Genomics of Drug Sensitivity in Cancer, respectively, and performs the Spearman’s correlation analysis to evaluate the association of gene expression with drug sensitivity. The positive correlation suggests that the elevated expression of an interesting gene is resistant to the drug and vice versa.

Statistical analysis

To compare the relative expression of the HPRT1 gene among different groups, we performed a Student’s $t$-test or one-way ANOVA tests as needed. Receiver operating characteristic (ROC) curves were illustrated to demonstrate the diagnostic utility of HPRT1 mRNA expression for patients with HNSCC. The area under the ROC curve (AUC) closer to 1.0 reflected that the test has the most diagnostic excellence. The maximum Youden index was used as a cutoff point. A $P$ value $<0.05$ was regarded as statistically significant. GraphPad Prism Software, version 9.0.0 (GraphPad Software Inc., La Jolla, CA, USA), was used to perform statistical analysis.

Results

The mRNA expression levels of the HPRT1 gene in HNSCC

We first investigated the transcript expression profiles of the HPRT1 gene. The RNA sequencing data retrieved from the UCSC Xena browser revealed that the mRNA expression levels of the HPRT1 gene were higher in HNSCC samples when compared with normal tissues ($P < 0.0001$; Fig. 1A). This was in accordance with our findings obtained from the GSE107591 dataset ($P = 0.022$; Fig. 1B). To confirm the results of our data mining, we assessed mRNA expression levels of the HPRT1 gene in 90 samples (45 from HNSCC tissues and 45 from adjacent normal tissues) using quantitative real-time PCR analysis. The results concluded that the transcript levels of the HPRT1 gene were apparently elevated in HNSCC compared with adjacent normal tissues ($P < 0.0001$; Fig. 1C).

The relationship between the HPRT1 mRNA expression and clinicopathological characteristics of patients with HNSCC

We further assessed the association of HPRT1 mRNA expression levels with clinicopathological features of patients in TCGA–HNSCC and our validation
cohorts. As presented in Fig. 2A–H, data determined that in comparison with normal control counterparts, the expression levels of the *HPRT1* gene were higher in patients with HNSCC with different age, sex, pathological stage and histological grade. However, further analysis indicated the absence of remarkable variation in mRNA expression levels of the *HPRT1* gene for patients with HNSCC of different ages (Fig. 2A,E), different sexes (Fig. 2B,F), different stages (Fig. 2C,G) and different grades (Fig. 2D,H).

The diagnostic utility of *HPRT1* mRNA expression for HNSCC

We appraised the diagnostic merit of *HPRT1* mRNA expression for HNSCC through the calculation of the AUCs. The findings demonstrated the diagnostic capacity of *HPRT1* mRNA expression in patients with HNSCC in TCGA cohort [AUC = 0.901, *P* < 0.0001, 95% confidence interval (CI) = 0.865–0.937; Fig. 3A], GSE107591 cohort (AUC = 0.701, *P* < 0.019, 95% CI = 0.542–0.859; Fig. 3B), and our validation cohort (quantitative real-time PCR data) (AUC = 0.701, *P* < 0.019, 95% CI = 0.542–0.859; Fig. 3C).

![Fig. 2. The association between the *HPRT1* mRNA expression levels and clinicopathological features. The correlation of *HPRT1* mRNA expression levels with patient’s age (A), sex (B), pathological stage (C) and histological grade (D) in the TCGA–HNSCC cohort. The relationship between the *HPRT1* mRNA expression levels and patient’s age (E), sex (F), pathological stage (G) and histological grade (H) in our validation cohort (quantitative real-time PCR data). Notably, the same normal datasets (*n* = 44 for TCGA–HNSCC cohort and *n* = 45 for validation cohort) have been used multiple times, in A–D and E–H, respectively. All data are shown as the mean ± standard deviation. *P* < 0.05 was regarded as statistically significant.]

![Fig. 3. The diagnostic value of *HPRT1* mRNA expression in HNSCC using ROC curves analysis. ROC curves for the TCGA cohort (A). ROC curves for the GSE107591 cohort (B). ROC curves for the validation cohort (quantitative real-time PCR data) (C).]
Prognostic value of HPRT1 mRNA expression for HNSCC

We evaluated the prognostic value of HPRT1 mRNA expression for HNSCC using the Kaplan–Meier Plotter database. The statistics suggested that higher levels of HPRT1 transcripts were correlated with the poor OS ($P = 1.3 \times 10^{-5}$; Fig. 4A). However, there was no significant difference between relapse-free survival (RFS) in patients with HNSCC with high expression levels of HPRT1 mRNA compared with those with low expression levels ($P = 0.22$; Fig. 4B).

The expression levels of the HPRT1 protein in HNSCC

To check the expression levels of the HPRT1 gene at protein levels, we then acquired the relevant expression data using the IHC staining images from the HPA database. The findings showed negative HPRT1 staining in normal oral mucosa of one patient, and moderate and strong HPRT1 staining in tumor tissues of one and three patients with HNSCC, respectively (Fig. 5A–E). Regarding the distribution, the expression of the HPRT1 protein is mainly noticed in the cytoplasm and cell membrane (Table 4).

Mutation analysis of the HPRT1 gene

The mutations of the HPRT1 gene in HNSCC samples were examined by the COSMIC database. The pie chart depicted that among four mutations in the HPRT1 gene, three were missense (75.00%) and one was synonymous (25.00%) substitutions (Fig. 6A). There were 50.00% A>G, 25.00% G>A and 25.00% G>C mutations in the HPRT1 gene coding region (Fig. 6B). We also explored the types of genetic alterations in the HPRT1 gene and their frequencies in 496 samples from the TCGA–HNSCC cohort using the cBioPortal database. As shown in Fig. 6C, the HPRT1 gene was altered in 7 (1%) cases of HNSCC, and the amplification was responsible for most changes. Moreover, using the “Survival” tab, we investigated the relationship between genetic alterations of the HPRT1 gene and survival times in patients with HNSCC. The Kaplan–Meier plot and log rank test uncovered that genetic alterations of the HPRT1 gene were not associated with the disease-specific survival ($P = 0.666$; Fig. 6D), disease-free survival ($P = 0.194$; Fig. 6E), progression-free survival ($P = 0.300$; Fig. 6F) and OS ($P = 0.130$; Fig. 6G) of patients with HNSCC.

Demonstration of the dysregulated coexpressed genes of the HPRT1 gene

To explore the potential roles of the HPRT1 gene in HNSCC, we first collected the coexpressed genes of the HPRT1 in HNSCC via the UALCAN database. Our data mining unveiled that 1966 genes were correlated with HPRT1 in HNSCC. Figure 7A,B shows the top 20 genes with positive and negative correlation with HPRT1 in HNSCC, respectively. When we assessed the expression value of the acquired genes in the GEPIA2 database, we found that only 268 genes

Fig. 4. The prognostic significance of HPRT1 mRNA expression in HNSCC using the data from the Kaplan–Meier Plotter database. The association between the HPRT1 mRNA expression and OS in patients with HNSCC (A). The relationship between the HPRT1 mRNA expression and RFS in patients with HNSCC (B). A log rank $P < 0.05$ was considered statistically significant.
were differentially expressed in HNSCC. Among them, IGF2BP2, ENO2, NETO2, HOXA10 and MYBL2 were the top five up-regulated (Fig. 7C), and SPINK5, SLURP1, HOPX, CRCT1 and CNFN were the top five down-regulated coexpressed genes of the HPRT1 gene in HNSCC (Fig. 7D).

**GO and KEGG pathway analysis**

Subsequently, we applied the Enrichr database to conduct the GO and KEGG pathway analysis for dysregulated coexpressed genes of the HPRT1 gene in HNSCC. The GO analysis unearthed that these genes

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**Table 4.** The IHC staining data of HPRT1 protein in one oral mucosa and four HNSCC tissues based on the HPA database.

| Patient parameters | Staining with antibody CAB012200 |
|--------------------|----------------------------------|
| ID; status         | Staining | Intensity | Quantity | Location                      |
| 1711; normal       | Male     | 71        | Not detectable | Negative | None | None |
| 2608; HNSCC        | Male     | 51        | Medium     | Moderate | >75% | Cytoplasmic/membranous |
| 1743; HNSCC        | Male     | 62        | High       | Strong   | >75% | Cytoplasmic/membranous |
| 1176; HNSCC        | Female   | 50        | High       | Strong   | >75% | Cytoplasmic/membranous |
| 773; HNSCC         | Female   | 59        | High       | Strong   | 25% to 75% | Cytoplasmic/membranous |

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**Fig. 5.** The proteomic analysis of HPRT1 expression in HNSCC using the data from the HPA database. The undetectable expression of HPRT1 protein in normal oral mucosa of a 71-year-old male patient, Patient ID: 1711 (A). The medium expression of HPRT1 protein in tumor tissue of a 51-year-old male patient with HNSCC, Patient ID: 2608 (B). The high expression of HPRT1 protein in tumor tissue of a 62-year-old male patient with HNSCC, Patient ID: 1743 (C). A 50-year-old female patient with HNSCC, Patient ID: 1176 (D). A 59-year-old female patient with HNSCC, Patient ID: 773 (E). Scale bars, 200 and 50 μm. HR, hazard ratio.
were enriched in centromere complex assembly (GO:0034508), chromatin remodeling at centromere (GO:0031055), mitotic sister chromatid segregation (GO:0000070), CENP-A containing nucleosome assembly (GO:0034080), CENP-A containing chromatin organization (GO:0061641), regulation of attachment of spindle microtubules to kinetochore (GO:0051988), histone exchange (GO:0043486), sister chromatid segregation (GO:0000819), regulation of metaphase/anaphase transition of cell cycle (GO:1902099) and mitotic

Fig. 6. The mutation analysis for the HPRT1 gene in HNSCC. The distribution and substitutions of mutations in the coding strand of the HPRT1 gene in HNSCC are based on the COSMIC database (A, B). The genetic alterations of the HPRT1 gene and their frequencies in patients with HNSCC according to the cBioPortal database (C). The association between the genetic alterations of the HPRT1 gene and disease-specific survival (DSS) in patients with HNSCC using the data from the cBioPortal database (D). The relationship between the genetic alterations of the HPRT1 gene and disease-free survival (DFS) in patients with HNSCC using the data from the cBioPortal database (E). The correlation between genetic alterations of the HPRT1 gene and progression-free survival (PFS) in patients with HNSCC obtained from the cBioPortal database (F). The association between the genetic alterations of the HPRT1 gene and OS in patients with HNSCC was retrieved from the cBioPortal database (G). A log rank $P < 0.05$ was considered statistically significant.
spindle elongation (GO:0000022) in BP (Table 5); and in histone kinase activity (GO:0035173), histone serine kinase activity (GO:0035174), 3'-5' DNA helicase activity (GO:0043138), DNA clamp loader activity (GO:0003689), protein-DNA loading ATPase activity (GO:0033170), DNA helicase activity (GO:0003678), single-stranded DNA-dependent ATPase activity (GO:0043142), peptide disulfide oxidoreductase activity (GO:0015037), DNA-dependent ATPase activity (GO:0008094) and four-way junction DNA binding (GO:0004040) in molecular function (Table 6); and in condensed nuclear chromosome kinetochore (GO:0000778), condensed chromosome, centromeric region (GO:0000779), condensed nuclear chromosome,
The top 10 GO molecular function terms for the HPRT1-correlated genes with differential expression in HNSCC.

| Term                                                      | P value | Adjusted P value | Combined score |
|-----------------------------------------------------------|---------|------------------|----------------|
| Histone kinase activity (GO:0035173)                      | 5.11E−8 | 3.05E−6          | 1568.152       |
| Histone serine kinase activity (GO:0035174)               | 1.09E−6 | 3.59E−5          | 1363.233       |
| 3’→5’ DNA helicase activity (GO:0043138)                  | 5.00E−7 | 2.13E−5          | 677.475        |
| DNA clamp loader activity (GO:0003689)                    | 8.09E−5 | 1.10E−3          | 524.070        |
| Protein–DNA loading ATPase activity (GO:0003170)          | 8.09E−5 | 1.10E−3          | 524.070        |
| DNA helicase activity (GO:0003678)                        | 2.39E−9 | 2.32E−7          | 436.665        |
| Single-stranded DNA helicase (GO:0043142)                 | 1.46E−5 | 3.10E−4          | 414.475        |
| DNA-dependent ATPase activity (GO:0043142)                | 1.90E−4 | 2.36E−3          | 317.626        |
| Peptide disulfide oxidoreductase activity (GO:0015037)    | 3.11E−9 | 2.32E−7          | 269.318        |
| DNA-dependent ATPase activity (GO:0008094)                | 5.13E−5 | 8.49E−4          | 245.0186       |

The top 10 GO CC terms for the HPRT1-correlated genes with differential expression in HNSCC.

| Term                                                      | P value | Adjusted P value | Combined score |
|-----------------------------------------------------------|---------|------------------|----------------|
| Condensed nuclear chromosome kinetochore (GO:000778)      | 2.32E−11| 3.52E−10         | 3226.434       |
| Condensed chromosome, centromeric region (GO:000779)      | 2.24E−16| 9.35E−15         | 2369.685       |
| Condensed nuclear chromosome, centromeric region (GO:000780) | 4.83E−9 | 5.38E−8          | 1436.064       |
| Spindle midzone (GO:0051233)                               | 9.25E−14| 2.20E−12         | 1327.506       |
| Condensed chromosome kinetochore (GO:000077)               | 6.42E−11| 8.93E−10         | 1289.637       |
| Chromosome, centromeric region (GO:000775)                 | 6.03E−17| 3.36E−15         | 1277.699       |
| Spindle microtubule (GO:0005876)                           | 2.00E−15| 6.69E−14         | 1045.629       |
| Spindle microtubule (GO:0005819)                           | 3.11E−26| 5.20E−24         | 1008.086       |
| Alpha DNA polymerase: primase complex (GO:0005658)         | 4.67E−5 | 2.79E−4          | 739.5584       |
| Chromosomal region (GO:0098887)                            | 3.86E−15| 1.07E−13         | 688.1082       |

Drug sensitivity analysis

We investigated the correlation between the expression profile of the HPRT1 gene with drug sensitivity through the GSCA-Lite database. The results have suggested that patients with cancer with high expression of the HPRT1 gene probably were sensitive to tozasertib, teniposide, manumycin A, clofarabine, GSK-J4, COL-3, CD-437, BRD-K01737880 and 3-Cl-AHPC and resistant to abiraterone (Fig. 8).
The aberrant expression of the HPRT1 gene has been documented in several cancers [15]. Nevertheless, the relationship between the HPRT1 gene and HNSCC is unknown. To our knowledge, our report is the first to investigate the expression of the HPRT1 gene at transcriptomic and proteomic levels, as well as its genetic alterations, diagnostic and prognostic merit, and probable functions in the head and neck squamous cancer. We hope that the findings of the present research advance the current knowledge, enhance therapeutic targets and strengthen the accuracy of diagnosis and prognosis for patients with HNSCC.

This study initially employed the data from the TCGA-HNSCC cohort and GSE107591 dataset to demonstrate the HPRT1 mRNA expression in HNSCC. The results showed that the HPRT1 mRNA expression was significantly up-regulated in cancer tissues compared with healthy control samples, which was consistent with the findings of our quantitative real-time PCR analysis for 45 paired HNSCC and normal tissues. We also assessed the expression levels of the HPRT1 gene in patients with HNSCC with different sex, age, pathological stage and histological grades in TCGA–HNSCC and our validation cohorts. The sex-based and age-based differences have contributed to the clinical presentations and prognosis of patients.

**Table 8.** The top 10 KEGG terms for the HPRT1-correlated genes with differential expression in HNSCC.

| Term                        | P value  | Adjusted P value | Combined score |
|-----------------------------|----------|------------------|----------------|
| DNA replication             | 9.47E−13 | 7.15E−11         | 930.412        |
| Cell cycle                  | 3.18E−16 | 4.81E−14         | 540.898        |
| Homologous recombination    | 6.28E−8  | 3.16E−6          | 303.411        |
| Fanconi anemia pathway      | 5.88E−7  | 1.27E−5          | 188.182        |
| p53 signaling pathway       | 5.12E−7  | 1.27E−5          | 156.536        |
| Progesterone-mediated       | 9.41E−8  | 3.56E−6          | 153.973        |
| oocyte maturation           |          |                  |                |
| Mismatch repair             | 2.32E−4  | 3.50E−3          | 131.078        |
| Oocyte meiosis              | 1.26E−7  | 3.79E−6          | 128.813        |
| One carbon pool by folate   | 2.32E−3  | 3.18E−2          | 79.364         |
| Cellular senescence         | 6.36E−5  | 1.20E−3          | 48.705         |

**Discussion**

The HPRT1 aberrant expression has been documented in several cancers [15]. Nevertheless, the relationship between the HPRT1 gene and HNSCC is unknown. To our knowledge, our report is the first to investigate the expression of the HPRT1 gene at transcriptomic and proteomic levels, as well as its genetic alterations, diagnostic and prognostic merit, and probable functions in the head and neck squamous cancer. We hope that the findings of the present research advance the current knowledge, enhance therapeutic targets and strengthen the accuracy of diagnosis and prognosis for patients with HNSCC.

This study initially employed the data from the TCGA–HNSCC cohort and GSE107591 dataset to demonstrate the HPRT1 mRNA expression in HNSCC. The results showed that the HPRT1 mRNA expression was significantly up-regulated in cancer tissues compared with healthy control samples, which was consistent with the findings of our quantitative real-time PCR analysis for 45 paired HNSCC and normal tissues. We also assessed the expression levels of the HPRT1 gene in patients with HNSCC with different sex, age, pathological stage and histological grades in TCGA–HNSCC and our validation cohorts. The sex-based and age-based differences have contributed to the clinical presentations and prognosis of patients.

**Fig. 8.** The drug sensitivity analysis for the HPRT1 gene. The data from the GSCAlite database represent the correlation of the HPRT1 gene expression and drug sensitivity. The negative correlation determines that patients with cancer with overexpression of the HPRT1 gene are sensitive to the drug and vice versa.
with HNSCC, respectively [32–35]. The sex-based analysis for both cohorts indicated the significant overexpression of HPRT1 gene level in tumor samples of both sexes compared with the normal tissues. However, there were no statistically significant differences between transcript levels of the HPRT1 gene in cancerous tissues of males and females. Besides, we observed no significant changes in the HPRT1 mRNA expression levels in the tumor tissues of patients with HNSCC of different ages in both cohorts. These may reflect that the patients’ sex and age probably did not influence the expression levels of HPRT1 mRNA in cancer tissues. We also discovered that the HPRT1 mRNA expression levels were remarkably elevated in advanced pathological stages and histological grades of HNSCC tissues in comparison with healthy control subjects in both cohorts. These observations suggested that the elevated expression levels of the HPRT1 gene may take part in the pathogenesis of HNSCC. The IHC staining images from the HPA database have revealed the medium and high expression levels of the HPRT1 protein in HNSCC tissues. Altogether, these findings unearthed that expression of the HPRT1 gene was significantly increased in HNSCC tissues.

One of the most difficult challenges about patients with HNSCC is the time and procedure of screening and diagnosis. It has been declared that the delayed diagnosis and treatment of patients with HNSCC aggravate the prognosis outcomes and increase the undesired morbidity and mortality of patients with cancer. Hence findings of new biomarkers would boost the clinical outcomes of patients with HNSCC [36–39]. In the present research, we carried out the ROC curves analysis to appraise the diagnostic utility of the HPRT1 mRNA expression levels for HNSCC. The data from TCGA (AUC = 0.901), GSE107591 (AUC = 0.701) and our validation cohort (AUC = 0.806) concluded that expression levels of the HPRT1 gene could be considered as a helpful marker for the diagnosis of patients with HNSCC. Moreover, the prognosis data obtained from the Kaplan–Meier Plotter disclosed that the higher expression levels of the HPRT1 gene in cancer tissues were significantly correlated with inferior survival time of patients with HNSCC, in line with the findings of previous studies that suggested the dysregulation of the HPRT1 expression was associated with poor survival outcomes of breast and endometrial cancers [17,18]. In summary, these results may indicate the great value of the HPRT1 tissue expression for diagnosis and risk stratification of patients with HNSCC.

It is widely accepted that the accumulation of genetic alterations plays a causal role in the tumorigenesis of HNSCC [40]. The further assessments using the COSMIC and cBioPortal databases unveiled that genetic alterations of the HPRT1 gene were rare in patients with HNSCC (7/496), and the missense mutations were responsible for the most types of genetic aberration in the HPRT1 gene coding strand. Besides, the genetic alterations of the HPRT1 gene were not associated with survival outcomes of patients with HNSCC.

Finding dysregulated coexpressed genes of the HPRT1 gene in HNSCC may lead to understanding how HPRT1 and its correlated genes help the progression of HNSCC. In the present research, we uncovered that HPRT1 and its coexpressed genes that are differentially expressed in HNSCC probably were enriched in the DNA replication, cell cycle, homologous recombination, Fanconi anemia, p53 signaling and mismatch repair pathways. When we checked the included coexpressed genes and their expression value in DNA replication (RFC5, PRIM2, FEN1, POLA2, RNASEH2A, RFC4, MCM7, RPA3, POLE2, MCM3, MCM6), cell cycle (MCM7, PLK1, BUB1B, TTK, CDC6, CDC25C, CDC20, CCNA2, CCNB2, CCNB1, CDC45, CDK4, CHEK2, CHEK1, CDK2, CDK1, MCM3, MCM6, BUB1, MAD2L1), homologous recombination (BLM, RAD51, EME1, RPA3, XRCC3, RAD54L, BRCA1, RAD54B), Fanconi anemia (FANCI, BLM, RAD51, EME1, UBE2T, RPA3, FANCA, BRCA1), p53 signaling (CCNB2, CCNB1, RRM2, CDK4, CHEK2, CHEK1, CDK2, CDK1, GTSE1) and mismatch repair (RFC5, RFC4, EXO1, RPA3) pathways, we found that all of them were significantly up-regulated in HNSCC. Previous studies have documented the homologous recombination and Fanconi anemia pathways deficiency in HNSCC [41,42]. Besides, further investigations showed several genes in DNA replication, and cell-cycle pathways were duplicated in the p53 signaling (CCNB2, CCNB1, CDK4, CHEK2, CHEK1, CDK2, CDK1) and mismatch repair (RFC5, RFC4, RPA3) pathways. Moreover, several pieces of evidence have reported that up-regulation of DNA replication and cell-cycle pathways genes in HNSCC [43–45]. Others have proposed that overexpression of the RRM2, GTSE1 and EXO1 genes in HNSCC have active roles in tumor progression and inhibition of the apoptosis pathway [46–49]. Some researchers have suggested that the HPRT1 gene, as a crucial component of the purine salvage pathway, plays a role in mediating the proliferation, autophagy and apoptosis-related processes in cancer cells [50–52]. IGFBP2 and SPINK5 were the top up-regulated and down-regulated coexpressed genes of the HPRT1 gene in HNSCC. Deng et al. [53] have determined that
IGF2BP2 is a prognostic gene and has vital roles in the progression of HNSCC. Others also have recognized SPINK5 as a novel tumor suppressor that suppresses migration and invasion of HNSCC cells [54,55]. These findings imply that HPRT1 and its associated gene likely mediate the progression of HNSCC through activation of the DNA replication and cell cycle and inhibition of apoptosis. Finally, the data obtained from the GSCALite database indicated that patients with increased expression of the HPRT1 gene may not respond to treatment with abiraterone very well and hopefully are responsive to therapies that consist of tozasertib, teniposide, manumycin A, clofarabine, GSK-J4, COL-3, CD-437, BRD-K01737880 and 3-Cl-AHPC.

Our study had some limitations:

1 As we stated in the introduction, HNSCCs are heterogeneous and can develop at different locations, such as the oral cavity, pharynx and larynx [4]. When we aimed to classify patients with HNSCC of TCGA, GSE107591 and our validation cohorts into oral or laryngeal squamous cell carcinoma, the number of patients in each group was few, and we could not perform meaningful statistical analyses. Therefore, we decided to consider all of them as HNSCC.

2 Although the expression analysis for the TCGA cohort demonstrated the absence of significant differences in the expression levels of the HPRT1 gene for patients with HNSCC who were human papillomavirus (HPV) positive in comparison with those with HPV negative (data not shown), we could not find any data about the HPV history of patients with HNSCC in GSE107591 and our validation cohorts.

3 The samples in the HPA database were few.

4 Although we determined that the expression levels of the HPRT1 gene were increased in HNSCC, the underlying mechanisms for this phenomenon remained unclear.

5 In this report, we uncovered that the HPRT1 gene and its dysregulated coexpressed genes likely mediate HNSCC progression through the cell cycle and apoptosis-related pathways. However, the exact function of the HPRT1 gene in these pathways remained ambiguous.

6 Except for mRNA expression analysis, other findings were not confirmed with reliable laboratory analysis, and we just focused on bioinformatics investigations. Relying on bioinformatics assessments may induce deviations.

Therefore, additional studies with appropriate sample size are required to explore: (a) the expression pattern of the HPRT1 gene in HNSCCs, such as oral squamous cell carcinoma (OSCC) -and laryngeal squamous cell carcinoma (LSCC); (b) the association between the expression levels of the HPRT1 gene and HPV status of patients with HNSCC; (c) the potential molecular etiologies for up-regulation of the HPRT1 gene in HNSCC; and (d) the exact underlying mechanisms in which the HPRT1 gene promotes the progression of HNSCC.

Conclusions

Overall, the findings of this research indicated that overexpression of HPRT1 is significantly correlated with the progression of HNSCC. Besides, the observed up-regulation of the HPRT1 expression in tumor tissues may be a valuable biomarker for diagnosis, prognosis and targeted treatment of patients with HNSCC. However, further investigations and clinical trials are mandatory to elucidate the involvement of HPRT1 in HNSCC.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

NS collected the patient’s samples and laboratory materials. FH, MA and MEK performed the laboratory experiments. MA and NS conducted the data extraction and statistical analysis and initially drafted the paper. PM and NS contributed to the design of the study and supervised all investigations. NS, PM, SP, SR and LH edited the manuscript. MA, SP, NS, SMA and PM checked the final version of the manuscript. All authors have contributed to, read and approved the final manuscript for submission.

Data accessibility

The data of TCGA were obtained from the website of the UCSC Xena browser (https://xenabrowser.net/). The data of the GSE107591 dataset were downloaded from the website of the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The data of the validation
Overexpression of HPRT1 in head and neck cancer

M. Ahmadi et al.

References

1 Jia S, Zhang M, Li Y, Zhang L and Dai W (2020) MAGE-A11 expression predicts patient prognosis in head and neck squamous cell carcinoma. Cancer Manag Res 12, 1427–1435.
2 Hsieh JC-H, Wang H-M, Wu M-H, Chang K-P, Chang P-H, Liao C-T and Liu C-T (2019) Review of emerging biomarkers in head and neck squamous cell carcinoma in the era of immunotherapy and targeted therapy. Head Neck 41, 19–45.
3 Economopoulou P, de Bree R, Kotsantis I and Psyrri A (2019) Diagnostic tumor markers in Head and Neck Squamous Cell Carcinoma (HNSCC) in the clinical setting. Front Oncol 9.
4 Alsahafi E, Begg K, Amelio I, Raulf N, Lucarelli P, Sauter T and Tavassoli M (2019) Clinical update on head and neck cancer: molecular biology and ongoing challenges. Cell Death Dis 10, 540.
5 Fan G, Tu Y, Wu N and Xiao H (2020) The expression profiles and prognostic values of HSPs family members in Head and neck cancer. Cancer Cell Int 20, 220.
6 Jin Y and Yang Y (2019) Bioinformatics-based discovery of PYGM and TNNC2 as potential biomarkers of head and neck squamous cell carcinoma. Biosci Rep 39.
7 Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D (2011) Global cancer statistics. CA Cancer J Clin 61, 69–90.
8 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68, 394–424.
9 Jiang P, Zhang Y, J Archibald S and Wang H (2015) Adaptive cell transfer after chemotherapy enhances survival in patients with resectable HNSCC. Int Immunopharmacol 28, 208–214.
10 Payne K, Brooks J, Spruce R, Batis N, Taylor G, Nankivell P and Mehanna H (2019) Circulating tumour cell biomarkers in head and neck cancer: current progress and future prospects. Cancers 11, 1115.
11 Chen Y-T, Yao J-N, Qin Y-T, Hu K, Wu F and Fang Y-Y (2018) Biological role and clinical value of miR-99a-5p in head and neck squamous cell carcinoma (HNSCC): a bioinformatics-based study. FEBS Open Bio 8, 1280–1298.
12 Monnat RJ Jr, Chiaverotti TA, Hackmann AF and Maresh GA (1992) Molecular structure and genetic stability of human hypoxanthine phosphoribosyltransferase (HPRT) gene duplications. Genomics 13, 788–796.
13 Torres RJ and Puig JG (2007) Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency: Lesch-Nyhan syndrome. Orphanet J Rare Dis 2, 48.
14 Townsend MH, Felsted AM, Ence ZE, Piccolo SR, Robison RA and O’Neill KL (2017) Elevated expression of hypoxanthine guanine phosphoribosyltransferase within malignant tissue. Cancer Clin Oncol 6, 19–34.
15 Passey MH, Felsted AM, Ence ZE, Piccolo SR, Neill KL and Robison RA (2018) Abstract 2536: Unique HPRT1 upregulation in malignant tissue: potential use as diagnostic biomarker. Cancer Res 78, 2536.
16 Townsend MH, Felsted AM, Burrup W, Robison RA and O’Neill KL (2018) Examination of hypoxanthine guanine phosphoribosyltransferase as a biomarker for colorectal cancer patients. Mol Cell Oncol 5, e1481810.
17 Townsend MH, Ence ZE, Felsted AM, Parker AC, Piccolo SR, Robison RA and O’Neill KL (2019) Potential new biomarkers for endometrial cancer. Cancer Cell Int 19, 19.
18 J Sedano M, I Ramos E, Choudhari R, L Harrison A, Subramani R, Lakshmanawamy R, Zilaie M and Gadad SS (2020) Hypoxanthine phosphoribosyl transferase 1 is upregulated, predicts clinical outcome and controls gene expression in breast cancer. Cancers 12, 1522.
19 Goldman M, Craft B, Zhu J and Haussler D (2017) The UCSC Xena System for Cancer Genomics Data Visualization and Interpretation. AACR.
20 Barrett T, Troup DB, Wilhite SE, Ledoux P, Rudnev D, Evangelista C, Kim IF, Soboleva A, Tomashevsky M and Edgar R (2007) NCBI GEO: mining tens of millions of expression profiles—database and tools update. Nucleic Acids Res 35, D760–D765.
21 Verduci L, Ferraiuolo M, Sacconi A, Ganci F, Vitale J, Colombo T, Paci P, Strano S, Macino G, Rajewsky N et al. (2017) The oncogenic role of circPVT1 in head and neck squamous cell carcinoma is mediated through the mutant p53/YAP/TEAD transcription-competent complex. Genome Biol 18, 237.
22 Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A (2015) Tissue-based map of the human proteome. Science 347, eaal3321.
23 Thul PJ, Åkesson L, Wiking M, Mahdessian D, Gelladaki A, Ait Blal H, Alm T, Asplund A, Björk L, Breckels LM et al. (2017) A subcellular map of the human proteome. Science 356, eaao1321.
24 Uhlen M, Zhang C, Lee S, Sjöstedt E, Fagerberg L, Bidkhori G, Benfetahs R, Arif M, Liu Z, Edfors F et al. (2017) A pathology atlas of the human cancer transcriptome. Science 357, eaau2507.
25 Györffy B, Lanczky A, Eklund AC, Denkert C, Budczies J, Li Q and Szallasi Z (2010) An online cohort used for this research are available from the corresponding author upon request.
survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Res Treat* **123**, 725–731.

26 Györffy B, Surowiak P, Budczies J and Lánczky A (2013) Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer. *PLoS One* **8**, e82241.

27 Forbes SA, Beare D, Bindal N, Bamford S, Ward S, Cole CG, Jia M, Kok C, Boutsolakis H, De T et al. (2016) COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res* **45**, D777–D783.

28 Wu P, Heins ZJ, Muller JT, Katsnelson L, de Bruijn I, Forbes SA, Beare D, Bindal N, Bamford S, Ward S, Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Kuleshov MV, Jones MR, Rouillard AD, Fernandez Hoang T, Zhang C, Geye HM, Speer T, Yu M, Hollows R, Wei W, Cazier JB, Mehanna H, Parry G, Massa ST, Cass LM, Challapalli S, Zahirsha Z, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E et al. (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* **6**, p1l.

29 Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E et al. (2012) The cBio Cancer Genomics Portal: an open platform for exploring multidimensional cancer genomics and clinical profiles using the cBioPortal. *Nucleic Acids Res* **40**, W90–W97.

30 Hoang T, Zhang C, Geye HM, Speer T, Yu M, Wiederholt PA, Kakusje JC, Traynor AM, Cleary JF, Dailey SH et al. (2012) Gender associations in head and neck squamous cell carcinoma (HNSCC). *J Clin Oncol* **30**, e16010.

31 Massa ST, Cass LM, Challapalli S, Zahirsha Z, Simpson M, Ward G and Osazuwa-Peters N (2019) Demographic predictors of head and neck cancer survival differ in the elderly. *Laryngoscope* **129**, 146–153.

32 Qadir F, Lalli A, Dar HH, Hwang S, Aldehlawi H, Ma H, Dai H, Waseem A and Teh M-T (2019) Clinical correlation of opposing molecular signatures in head and neck squamous cell carcinoma. *BMC Cancer* **19**, 830.

33 Hollows R, Wei W, Cazier JB, Mehanna H, Parry G, Halford G and Murray P (2019) Association between loss of Y chromosome and poor prognosis in male head and neck squamous cell carcinoma. *Head Neck* **41**, 993–1006.

34 Schutte HW, Heutink F, Wellenstein DJ, van den Broek GB, van den Hoogen FJA, Marres HAM, van Herpen CML, Kaanders JHAM, Merkx TMAW and Takes RP (2020) Impact of time to diagnosis and treatment in head and neck cancer: a systematic review. *Otalaryngol Head Neck Surg* **162**, 446–457.

35 Coca-Pelaz A, Takes RP, Hutcheson K, Saba NF, Haigentz M, Bradford CR, de Bree R, Strojan P, Lund VJ, Mendenhall WM et al. (2018) Head and neck cancer: a review of the impact of treatment delay on outcome. *Adv Ther* **35**, 153–160.

36 Li Q, Shen Z, Wu Z, Shen Y, Deng H, Zhou C and Liu H (2020) High P4HA1 expression is an independent prognostic factor for poor overall survival and recurrent-free survival in head and neck squamous cell carcinoma. *J Clin Lab Anal* **34**, e23107.

37 Budach V and Tinhofer I (2019) Novel prognostic clinical factors and biomarkers for outcome prediction in head and neck cancer: a systematic review. *Lancet Oncol* **20**, e313–e326.

38 Puram SV, Tirosch I, Parikh AS, Patel AP, Yizhak K, Gillespie S, Rodman C, Luo CL, Mroz EA, Emerick KS et al. (2017) Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. *Cell* **171**, 1611–1624.e24.

39 Rieke DT, Ochsenreither S, Klinghammer K, Seiwert TY, Klauschen F, Tinhofer I and Keilholz U (2016) Methylation of RAD51B, XRCC3 and other homologous recombination genes is associated with expression of immune checkpoints and an inflammatory signature in squamous cell carcinoma of the head and neck, lung and cervix. *Oncotarget* **7**, 75379–75393.

40 Romick-Rosendale LE et al. (2016) Defects in the Fanconi anemia pathway in head and neck cancer cells stimulate tumor cell invasion through DNA-PK and Ruc1 signaling. *Clin Cancer Res* **22**, 2062–2073.

41 Rothenberg SM and Ellison LW (2012) The molecular pathogenesis of head and neck squamous cell carcinoma. *J Clin Invest* **122**, 1951–1957.

42 Ansari SS, Sharma AK, Zepp M, Ivanova E, Bergmann F, König R and Berger MR (2017) Upregulation of cell cycle genes in head and neck cancer patients may be antagonized by erufosine’s down regulation of cell cycle processes in OSCC cells. *Oncotarget* **8**, 5797–5810.

43 van Harten AM, Buizje M, van der Mast R, Rooimans MA, Martens-de Kemp SR, Bachas C, Brink A, Stiger-van Walsum M, Wolkhuiss RMF and Brakenhoff RH (2019) Targeting the cell cycle in head and neck cancer by Chk1 inhibition: a novel concept of bimodal cell death. *Oncogenesis* **8**, 38.

44 Roh V, Hiu-Feige A, Misetic V, Rivals J-P, Sponarova J, Teh M-T, Ferreira Lopes S, Truan Z, Mermod M, Monnier Y et al. (2020) The transcription factor FOXM1 regulates the balance between proliferation and aberrant differentiation in head and neck squamous cell carcinoma. *J Pathol* **250**, 107–119.

45 Wang S, Wang XL, Wu ZZ, Yang QC, Xiong HG, Xiao Y, Li H and Sun ZJ (2021) Overexpression of
RRM2 is related to poor prognosis in oral squamous cell carcinoma. *Oral Dis* **27**, 204–214.

48 Dai Y, Tang Z, Yang Z, Zhang L, Deng Q, Zhang X, Yu Y, Liu X and Zhu J (2018) EXO1 overexpression is associated with poor prognosis of hepatocellular carcinoma patients. *Cell Cycle (Georgetown, Tex.)* **17**, 2386–2397.

49 Rahman MA, Amin ARMR, Wang D, Koenig L, Nannapaneni S, Chen Z, Wang Z, Sica G, Deng X, Chen Z et al. (2013) RRM2 regulates Bcl-2 in head and neck and lung cancers: a potential target for cancer therapy. *Clin Cancer Res* **19**, 3416–3428.

50 Camici M, Garcia-Gil M, Pesi R, Allegrini S and Tozzi MG (2019) Purine-metabolising enzymes and apoptosis in cancer. *Cancers* **11**, 1354.

51 Yin J, Ren W, Huang X, Deng J, Li T and Yin Y (2018) Potential mechanisms connecting purine metabolism and cancer therapy. *Front Immunol* **9**.

52 Villa E, Ali ES, Sahu U and Ben-Sahra I (2019) Cancer cells tune the signaling pathways to empower de novo synthesis of nucleotides. *Cancers* **11**, 688.

53 Deng X, Jiang Q, Liu Z and Chen W (2020) Clinical significance of an m6A reader gene, IGF2BP2, in head and neck squamous cell carcinoma. *Front Mol Biosci* **7**, 68.

54 Lv Z, Wu K, Qin X, Yuan J, Yan M, Zhang J, Wang L, Ji T, Cao W and Chen W (2020) A novel tumor suppressor SPINK5 serves as an independent prognostic predictor for patients with head and neck squamous cell carcinoma. *Cancer Manag Res* **12**, 4855–4869.

55 Jayakumar A, Chattopadhyay C, Wu H, Briggs K and Henderson Y (2014) LEKTI, a physiological inhibitor of multiple serine proteinases, blocks migration and invasion of Head and Neck Squamous Cell Carcinoma (HNSCC) cells. *MOJ Proteomics Bioinform* **1**, 00015.