Comprehensive analysis of the expression and prognosis for CDCAs in head and neck squamous cell carcinoma

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Abstract

Head and neck squamous cell carcinoma (HNSCC), a tumor included oral cavity, lips, larynx, oropharynx, and the nasopharynx et al. The cell division cycle-associated (CDCA) protein family (CDCA1-8) critical for normal cell function and cancer cell proliferation. We explored the mutation signatures and expression levels of various CDCAs in detail in HNSCC. A comprehensive bioinformatics analysis pipeline based on copy number and gene expressions data from patients with HNSCC in order to given new insights into the possible functions and distinct prognostics that underlie CDCA regulation. We compared the transcriptional expression of CDCAs in HNSCC and found significantly elevated mRNA expression of CDCA1-8 in HNSCC tissues across multiple datasets. We also found CDCA5/6/8 are over-expressed both transcriptionally and translationally in patients with HNSCC. Our results suggested that that mRNA levels of CDCA1/2/4/7 related to the prognosis and can be used as a new useful biomarker for predicting the survival of HNSCC patients. The top 5 CDCAs neighboring gene alterations in HNSCCs were found in MYC, STAG1, RAD21, KLHL9 and NDC80. Multivariable Cox proportional hazard model also showed that CD8+ T cells were higher (P<0.05) in HNSCC-HPV-pos patients and that this was related to CDCA1/2/3/4/5/7. This study utilizes online tools to conduct specific gene analyses from free open databases, but our study requires more large-scale genomics research and basic research.

Introduction

Head and neck squamous cell carcinoma (HNSCC), a tumor included oral cavity, lips, larynx, oropharynx, and the nasopharynx et al[1]. The tumor with a yearly incidence of over 650,000 new diagnosis and 90,000 decease worldwide[2]. The risk factors for HNSCC involve in smoking, alcohol drinking and virus infection, such as human papilloma virus (HPV)[3]. Unfortunately, there is insufficiency of symptoms at the early stage of the cancer, causing most patients with HNSCC to be diagnosed at the progressive stages. Consequently, the survival rate of 5-year is below 50% and patients that suffer from local recurrence and metastasis have an even
lower survival rate of 35%[4]. When in the advanced stage, therapeutics can affect organ structures function that related to swallowing and speaking, leading to a decline in the patient’s quality of life[5,6]. The occurrence of HNSCC is a complicated mechanism that involves multiple molecules. Ni et al[7] found that HPV and HPV16 DNA was detected in 26.4% and 71% of the 303 HNSCCs, respectively. Thus, prophylaxis against HPV infection may help reduce the incidence of this disease. A recent study proposed that zeste homolog 2 (EZH2) regulates epithelial-to-mesenchymal transition (EMT), metastasis and tumor invasion in HNSCC by regulating the STAT3/VEGFR2 axis[8]. Valenti et al[9] reported that miR-205-5p’s can impact genomic instability in HNSCC by selectively targeting the DNA damage response (DDR) genes RAD17 and BRCA1. In spite of the advances that have been made in the past decades, which include combining chemotherapy, radiation, and surgery, many patients still experience tumors recurrences and metastasis even received treatment, which leads to therapeutic failure[10].

The cell division cycle-associated (CDCA) protein family (CDCA1-8) not only necessary for normal cell function, but also plays a key role in cancer cell proliferation. Some studies have highlighted that abnormal expression of cell cycle regulatory proteins may cause cancer. Phan et al[11] found that CDCA3/5/8 are significantly higher in breast cancer tissue than control tissue, leading to a dramatic reduction in patient survival among breast cancer patients. A clinical trial that was now performed with castration resistant prostate cancer (CRPC) by a CDCA1 peptide vaccination was found to effectively induce peptide-specific CTLs for CRPC patients[12]. In addition, siRNA-mediated knockdown of CDCA1 in oral cavity carcinoma (OCC) tumor cells was found to induce a significant apoptotic response[13]. The CDCA1 protein family is often co-expressed with many other cell cycle regulators, involving CDC23/CDC7/CDC2/MCAK/MKI67 and topoisomerase II, to regulate tumor cell growth[14]. To date, the mechanism by which CDCAs are activated or deactivated in the development and progression of HNSCC still remains unclear. We explored the mutation signatures and expression levels of various CDCAs in detail using a comprehensive bioinformatics analysis pipeline based on copy number and gene expressions data from patients with HNSCC in order to offer more knowledge into the potential functions and distinct prognostics that underlie CDCAs regulation. We also discuss the opportunities and challenges in using these to derive clinical benefit for HNSCC patients.

Methods and materials

ONCOMINE database and Human Protein Atlas

The HNSCC mRNA expression data of CDCAs were obtained from the Oncomine[15], which is a database that involve 86,733 samples and 715 gene expression data sets. Oncomine as well the largest oncogene chip database as well as incorporated data mining database. This analysis was based on a number of prior HNSCC researches. The level of CDCAs was evaluated in HNSCC tissue and in control tissue. P<0.05 considered statistically significant. All the Data from Genomic Data Commons Data Portal. The Human Protein Atlas (HPA) is an online tool that included immunohistochemistry expression data for distribution and expression of proteins across 20 cancer tissues, 48 human normal tissues, 47 cell lines, and 12 blood cells[16]. We used immunohistochemistry images to directly compare protein expression of CDCAs among normal and cancer tissues.

GEPIA dataset and UALCANK analysis

GEPIA[17] is an interactive online database which allowed users to found RNA seq expression data or samples based on the Genotype Tissue Expression projects (GTEx) and The Cancer
Genome Atlas (TCGA). Meanwhile, GEPIA also offers customizable functions such as profiling based on pathological stage of cancer, type of cancer, survival analysis, correlation analysis and similar gene identification. UALCAN[18] is a website that helps analyze, integrate and discover cancer transcriptomic data and deep analyses of TCGA gene expression information. One of the portal’s highlight characteristics is that it can determine biomarkers or to perform in silico analysis of potential candidate genes of interest to assess expression in various subgroups, such as age, gender, race, and grade.

Kaplan-Meier plotter and cBioPortal
Kaplan-Meier plotter[19] was used to predicted the prognostic significance of different CDCAs in HNSCC. The database includes RNA-seq information based on TCGA and GEO. By setting different parameters, different subgroups can explore including patients with various pathologies, treatment ways, and data sets. The cBioPortal[20] is a free asset that can download large-scale cancer genomics data sets encompassing 245 cancer researches. Using cBioPortal to explored CDCAs genetic alterations in CDCAs. An interaction network of the CDCAs and the co-expressed genes were also analyzed. GO and KEGG functions of CDCAs mutations and top 50 genes that were obviously linked to CDCAs mutations were performed via DAVID online tool.

TIMER analysis
TIMER[21] is a useful tool for systematic found of immune infiltrates across different cancer types. Gene module can explore correlation among CDCAs and the abundance of immune infiltrates in HNSCC. The survival module was used to draw Kaplan-Meier plots for immune infiltrates and CDCAs for visualization of survival differences.

Results
High-expression of CDCAs family members
We first investigate the mRNA and protein expression of CDCAs using the ONCOMINE and HPA. We found obviously elevated expression of CDCA1-8 in HNSCC tissues (Fig 1). According to the Peng statistics[22], CDCAI expression is 1.982-fold higher in OCC tissues compared to normal samples (P = 3.03E-9), Pyeon[23] observed 6.027-fold increase in CDCAI across multiple HNSCC cancer samples (P = 4.64E-7), and Sengupta[24] found 4.267-fold in HNSCC tissues (P = 1.22E-5, Table 1). Pyeon[23] observed 1.974-fold increase in CDCA2 (P = 9.34E-6) and Sengupta[24] found a 2.490-fold increase in CDCA2 (P = 1.70E-6). Pyeon[23] observed 1.926-fold increase in CDCA3 (P = 4.16E-6). Data from Peng Head-Neck statistics[22] indicates that CDCAA is over-expressed in OCC tissues with a fold change of 1.580 (P = 3.76E-9), while Pyeon[23] observed 2.001-fold increase in CDCAA4 (P = 3.87E-10). In Peng statistics[22], CDCA5 was found in the OCC tissues with a fold change of 1.764 (4.16E-12), Pyeon[23] observed 2.668-fold increase in CDCA5 (P = 9.34E-6), Sengupta[24] found 2.053-fold increase in CDCA5 (P = 7.02E-7) and Ye[25] observed a 2.553-fold increase of CDCA5 in tongue tissue (P = 4.93E-9). Significant up-regulation of CDCA6 was also found in HNSCC tissues. In Sengupta[24], CDCA6 was found to high expressed with a fold change of 1.574 (P = 2.09E-5). According to Ye[25] statistics, CDCA6 was high expressed with a fold change of 1.728 (P = 3.66E-6). Sengupta[24] showed a 2.402-fold increase in CDCA7 (P = 1.22E-6). According to Giordano[26], CDCA8 found a fold change of 1.515 (P = 4.63E-5). Similarly, Pyeon[23] statistics indicate that CDCA8 with a fold change of 1.728 (P = 5.82E-7) and Peng statistics[22] observed a 1.607-fold in tumor samples (P = 1.41E-7).
We next analyzed the protein expression of CDCAs and the result indicated low protein expression of CDCA5/6/8 in normal tissues and high protein expression in tumor tissues. In addition, results also indicate medium expression of CDCA2 in normal tissues and high expression in tumor tissues.

**Table 1. The significant changes of CDCA expression in transcription level between different types of HNSCC and normal tissues (Oncomine database).**

| CDCAs          | Type of HNSCC† Cancer versus Normal HNSCC Tissue | Fold Change | P Value  | t Test | Source and/or Reference       |
|----------------|------------------------------------------------|-------------|----------|--------|--------------------------------|
| CDCA1          | Oral Cavity Squamous Cell Carcinoma              | 1.982       | 3.03E-9  | 6.934  | Peng Head-Neck statistics[22]  |
|                | Multi-cancer                                     | 6.027       | 4.64E-7  | 7.030  | Pyeon Multi-cancer[23]         |
|                | Nasopharyngeal Carcinoma                         | 4.267       | 1.22E-5  | 5.894  | Sengupta Head-Neck Statistics[24] |
| CDCA2          | Multi-cancer                                     | 1.974       | 9.34E-6  | 5.314  | Pyeon Multi-cancer[23]         |
|                | Nasopharyngeal Carcinoma                         | 2.490       | 1.70E-6  | 6.549  | Sengupta Head-Neck Statistics[24] |
| CDCA3          | Multi-cancer                                     | 1.926       | 4.16E-6  | 5.716  | Pyeon Multi-cancer[23]         |
| CDCA4          | Oral Cavity Squamous Cell Carcinoma              | 1.580       | 3.76E-9  | 6.636  | Peng Head-Neck statistics[22]  |
|                | Multi-cancer                                     | 2.001       | 3.87E-10 | 10.331 | Pyeon Multi-cancer[23]         |
| CDCA5          | Oral Cavity Squamous Cell Carcinoma              | 1.764       | 4.16E-12 | 8.049  | Peng Head-Neck statistics[22]  |
|                | Multi-cancer                                     | 2.268       | 9.34E-6  | 7.206  | Pyeon Multi-cancer[23]         |
|                | Nasopharyngeal Carcinoma                         | 2.055       | 7.02E-7  | 6.641  | Sengupta Head-Neck Statistics[24] |
| CDCA6          | Nasopharyngeal Carcinoma                         | 1.574       | 2.09E-5  | 4.697  | Sengupta Head-Neck Statistics[24] |
| CDCA7          | Nasopharyngeal Carcinoma                         | 2.402       | 1.22E-6  | 5.530  | Sengupta Head-Neck Statistics[24] |
| CDCA8          | Thyroid Gland                                    | 1.515       | 4.63E-5  | 7.129  | Giordano Thyroid Statistics[26] |
|                | Multi-cancer                                     | 1.822       | 5.82E-7  | 9.003  | Pyeon Multi-cancer[23]         |
|                | Oral Cavity Squamous Cell Carcinoma              | 1.607       | 1.41E-7  | 5.759  | Peng Head-Neck statistics[22]  |

†HNSCC: head and neck squamous cell carcinoma.

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expression in tumor tissues. Meanwhile, we observed no protein expression of CDCA4 in either normal or HNSCC tissues (HPA database missed CDCA1/3/7 data, Fig 2). Overall, our results suggest that CDCA5/6/8 are over-expressed both transcriptionally and translationally in patients with HNSCC.

**Clinical subgroup analysis**

We first using the GEPIA dataset to compared the expression of CDCAs among cancer and normal tissues. Our results indicate that the CDCA1/2/3/4/5/6/8 are significantly higher in HNSCC tissues (Fig 3). Next, we performed subgroup analysis of multiple clinical pathological features using the TCGA database. Subgroup analysis by age, indicated that transcriptional levels of CDCAs were higher in HNSCC patients when compared to healthy individuals.

![Representative immunohistochemistry images of distinct CDCAs family members in HNSCC tissues and normal tissues (Human Protein Atlas database).](https://doi.org/10.1371/journal.pone.0236678.g002)
Additionally, subgroup analysis by HPV status analysis; gender subgroup, and tumor grade demonstrated that CDCAs were significantly higher in HNSCC patients across all subgroups (Fig 4).

Prognostic analysis

Next, we tried to explore the prognostic significance of CDCAs in HNSCC patients, data for which was obtained from publicly available online datasets. The results are shown in Fig 5, which indicate that higher expression of CDCA4 (HR = 0.38, 95% CI: 0.19–0.85, P = 0.014) was related to longer relapse free survival (RFS). Higher expression of CDCA1 (HR = 0.71, 95% CI: 0.50–0.99, P = 0.043), CDCA2 (HR = 0.74, 95% CI: 0.56–0.99, P = 0.037) and CDCA7 (HR = 0.72, 95% CI: 0.52–0.99, P = 0.043) was also related to longer overall survival (OS). These results suggest that the levels of CDCA1/2/4/7 may play a key role in HNSCC prognosis.

Function analysis of CDCAs in HNSCC

We explored CDCAs alterations and networks using the cBioPortal. 50 neighboring genes that were found to be significantly linked to CDCAs mutations. Among the 528 HNSCC tumor samples that were sequenced, genetic alterations were found in 90 samples with a mutation rate of 18%. CDCA5 was ranked as the most mutated gene among CDCAs with mutation rates of 5%. We also showed the network for CDCAs and the 50 most frequently altered neighboring genes (Fig 6). The top 5 CDCAs neighboring gene alterations in HNSCCs were found in MYC, STAG1, RAD21, KLHL9 and NDC80 (Table 2).

Next, we analyzed the functions of CDCAs and these 50 genes using GO and KEGG (S1 Appendix). GO analysis indicate that changes in biological processes included enrichment in sister chromatid cohesion, cell division, mitotic nuclear division, gene silencing by RNA, and protein sumoylation among others. Molecular function was mainly enriched in protein heterodimerization activity, microtubule plus-end binding, protein phosphatase type 2A regulator activity, nucleocytoplasmic transporter activity, and protein binding. Changes in cell component were largely enriched in condensed chromosome kinetochore, chromosome, centromeric region, kinetochore, cytosol, nucleosome and others. Pathway enrichment analysis according to KEGG was mainly enriched in PI3K-Akt and AMPK signaling pathway, endometrial cancer, acute myeloid leukemia, colorectal cancer, central carbon metabolism in cancer, transcriptional misregulation in cancer, and chronic myeloid leukemia.

Immune infiltrates of CDCAs in HNSCC

There is a statistically significant correlation between CDCAs expression in HNSCC and abundance of immune infiltrates (P<0.05, Fig 7). We explored the difference in cumulative survival between HNSCC, HNSCC-HPV-pos and HNSCC-HPV-neg and found that the HNSCC-HPV-pos subgroup showed significantly higher B cells, CD8+ T cells and neutrophil immune infiltrates, (P<0.05) which was related to CDCAs levels. This indicates that these immune cell infiltrations significantly affect prognosis. Therefore, it is worth further

![Fig 3. The box plot expression of CDCAs in HNSCC (GEPIA database).](https://doi.org/10.1371/journal.pone.0236678.g003)
Fig 4. Boxplot showing relative expression of CDCAs in subgroups of patients with HNSCC, stratified based on gender, age, HPV status, gender and tumor grade (UALCAN).

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researching and exploring this association (Fig 8). Multivariable Cox proportional hazard model also showed that CD8+ T cells immune infiltrates were significant higher ($P<0.05$) in HNSCC-HPV-pos patients and that this was related to CDCA1/2/3/4/5/7 Table 3.
Discussion

Though certain CDCAs have been shown to play a critical role in tumor, the specific roles of CDCAs in HNSCC remains unclear. Thus, we first explored the mutational, gene expression, and prognostic landscape of various CDCAs in patients with HNSCC. We found higher mRNA expression across all CDCAs, and the expression of CDCAs was significantly linked to patients’ individual cancer stages. Moreover, we explored the immune status of HNSCC patients which can potentially help guide the development of novel therapies and to improve response to immunotherapy.

A growing number of studies have shown that CDCAs are highly expressed in tumors and have a role in regulating tumor cell cycle, promoting tumor cell proliferation, and reducing tumor cell apoptosis, which results in poor prognosis. CDCA1, also known as NUF2, codes for a protein that is essential for nuclear division and microtubule stabilization[27]. Tokuzum et al [27] reported that CDCA1-specific siRNA inhibits the cell proliferation of WM115 and SKMEL2 cells, but does not reduce the invasion activity or migration in malignant melanoma patients. Tomita et al[28] demonstrated that the existence of CDCA1-specific Th cell responses

| Gene Symbol | Amplification | Mutation | Total Alteration |
|-------------|---------------|----------|-----------------|
| MYC         | 12.1          | 1.2      | 13.2            |
| STAG1       | 8.9           | 1.0      | 9.7             |
| RAD21       | 8.3           | 1.0      | 9.3             |
| KLHL9       | 0.8           | 0.6      | 7.3             |
| NDC80       | 5.0           | 0.8      | 6.0             |

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in HNSCC patients underline the potential utility of CDCA1-LPs for propagation of both CDCA1-specific CTLs and Th cells. Similarly, Kaneko et al. [29] found that knockdown of CDCA1 and KNTC2 genes in colorectal cancer cells better inhibits tumor cell growth. Our results show that CDCA1 is highly expressed in HNSCC tissues, and CDCA1 is significantly correlated to patients’ survival and abundance of immune infiltrates. Moreover, our cumulative survival analyses show that CD8+ T cell immune infiltrates significantly affect the prognosis of these patients. Thus, it is worth further exploring this association.

CDCA2 is a nuclear protein that binds to protein phosphatase 1γ (PP1γ) and participated in DNA damage during cell cycle [30]. Moreover, CDCA2 modulates phosphorylation of the primary mitotic histone H3 in a PP1-dependent manner [31]. Some studies indicated that CDCA2 act for a very powerful prognostic marker for poor patient survival and malignancy in cancers such as neuroblastoma, lung adenocarcinoma, and oral squamous cell carcinoma tissue [32–34]. A recent study found that overexpression of CDCA2 may target CCND1 to promote colorectal cancer cell proliferation and tumorigenesis via activation of the PI3K/AKT pathway [35]. Interestingly, in our analysis of 50 neighbor genes that were significantly related to CDCAs mutations, the KEGG results showed a high enrichment of genes involved in the PI3K-Akt and AMPK signaling pathway. Thus, our study provides critical information that can be utilized for future studies.

CDCA3 is part of the SKP1-Cullin-RING-F-box (SCF) ubiquitin ligase (E3) complex, which degrades the endogenous cell cycle inhibitor WEE1, thereby regulating cell cycle [36]. CDCA3, through regulation by specificity protein 1 (SP1) and hypomethylation of its gene body, affects gastric cancer (GC) cell proliferation and invasion [37]. In addition, CDCA3 activated the Ras signaling pathway to facilitate cell proliferation in vitro and in vivo in GC cells [38]. Another study also found that FoxB3 can bind to the CDCA3 promoter region and transactivate CDCA3 expression to induce prostate cancer progression [39]. Our results show that HNSCC tissue highly express CDCA3. To date, however, no studies have investigated the connection between HNSCC and CDCA3 and more research is needed.

CDCA4, also known as HEPP/SEI-3/TRIP-Br3 is a target gene of transcription factor E2F, was discovered in 2001 and has shown to be related to the regulation of genes regulating the growth and differentiation of hematopoietic stem and progenitor cells [40]. Xu et al. [41] found that CDCA4 enhanced proliferation and reduced apoptosis in the MCF-7/ADM breast cancer cells in vitro. A recent study also suggested that CDCA4 may be involved in regulating triple
negative breast cancer (TNBC) progression\cite{42}. Results from our study indicate that CDCA1/2/4/7 may serve as novel biomarkers for prediction of HNSCC patients' survival. CDCA5 is a critical regulator of sister chromatid condensation and separation during cell division\cite{43}. CDCA5 could promote proliferation, migration, invasion, apoptosis resistance and decrease chemosensitivity to cisplatin in esophageal squamous cell carcinoma (ESCC) cells\cite{44}. Moreover, CDCA5 was shown to be upregulated in hepatocellular carcinoma (HCC) tissues compared to paracancerous tissues, is negatively correlated with patient survival and associated with cell abnormalities via upregulation of the AKT pathway\cite{45}. CDCA6 also known as CBX2, encodes a component of the polycomb multiprotein complex. CDCA6 depletion abrogated cell viability and induced caspase 3-mediated apoptosis in metastatic prostate cancer cell lines\cite{46}. One study also found that CDCA6 upregulation and amplification was significantly related to lower overall survival and metastatic progression across many cancer types\cite{47}. While our study shows high expression of CDCA6 in HNSCC tissues, though there is a paucity of studies in literature that have investigated this connection. Thus, there is a need to conduct research on the role of CDCA6 in HNSCC. CDCA7, also known as JP01, is considered to be a c-Myc target gene that is involved in c-Myc-mediated cell transformation\cite{48}. One study found that depletion of CDCA7 extremely minimize the tumorigenicity and colonization capacities of TNBC cells in vivo\cite{49}. Jenness et al reported that the HELLS-CDCA7 complex possesses nucleosome remodeling activity\cite{50}. Another study discovered a role for CDCA7 in Centromeric Instability and Facial Anomalies syndrome, a life-threatening immunodeficiency \cite{51}. In addition, AKT signaling to CDCA7 could alter MYC-dependent growth and transformation, contributing to tumorigenesis\cite{52}. 

Table 3. Multivariate survival model analysis based on TIMER online tool (HNSCC-HPVpos).

| Clinicopathologic variable | coef | HR  | 95% CI L  | 95% CI U  | p-Value | sig |
|----------------------------|------|-----|-----------|-----------|---------|-----|
| Age                        | -0.012 | 9.880E-01 | 0.908  | 1.076E+00 | 0.789   |     |
| Gender Male                | -0.153 | 8.580E-01 | 0.127  | 5.803E+00 | 0.875   |     |
| Race Black                 | 19.439 | 2.767E+08  | 0  | Inf  | 0.999   |     |
| Race White                 | 18.555 | 1.145E+08  | 0  | Inf  | 0.999   |     |
| Stage II                   | 17.660 | 4.671E+07  | 0  | Inf  | 0.998   |     |
| Stage III                  | 14.808 | 2.700E+06  | 0  | Inf  | 0.999   |     |
| Stage IV                   | 15.707 | 6.626E+06  | 0  | Inf  | 0.999   |     |
| Purity                     | -0.462 | 6.300E-01  | 0.010  | 3.942E+01 | 0.827   |     |
| B cells                    | 12.517 | 2.730E+05  | 0  | 1.380e+16 | 0.320   |     |
| CD+ 8 T cell               | -24.639 | 0  | 0  | 0  | 0.002   | ** |
| CD4+ T cells               | -10.185 | 0  | 0  | 2.371e+01 | 0.135   |     |
| Macrophages                | 17.151 | 2.808E+07  | 0.005  | 1.589E+17 | 0.134   |     |
| Neutrophils                | -10.292 | 0  | 0  | 2.450E+01 | 0.135   |     |
| Dendritic                  | 8.031 | 3.074E+03  | 0.014  | 6.843E+08 | 0.201   |     |
| NUF2                       | 2.975 | 1.960E+01  | 2.702  | 1.421E+02 | 0.003   | ** |
| CDCA2                      | -1.315 | 2.680E-01  | 0.080  | 9.010E-01 | 0.033   | * |
| CDCA3                      | -2.501 | 8.200E-02  | 0.018  | 3.680E-01 | 0.001   | ** |
| CDCA4                      | 1.834 | 6.260E+00  | 1.469  | 2.668E+01 | 0.013   | * |
| CDCA5                      | 2.282 | 9.800E+00  | 1.748  | 5.496E+01 | 0.009   | ** |
| CBX2                       | -0.300 | 7.410E-01  | 0.343  | 1.598E+00 | 0.444   |     |
| CDCA7                      | -1.141 | 3.190E-01  | 0.135  | 7.550E-01 | 0.009   | ** |
| CDCA8                      | 0.541 | 1.718E+00  | 0.151  | 1.950E+01 | 0.663   |     |

P-value Significant Codes: 0 ≤ *** < 0.001 ≤ ** < 0.01 ≤ * < 0.05 ≤ < 0.1.

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CDCAs, also known as Borealin/DasraB, encodes a component of the chromosomal passenger complex and is essential for chromatin-induced microtubule stabilization and spindle formation[53]. One study also reported that CDCA8 was significantly linked to poor prognosis in patients with cutaneous melanoma[54], breast cancer[55], colorectal cancers[56] and lung cancer[57]. Our results suggest that CDCAS/6/8 are higher expressed in patients with HNSCC, both transcriptionally and translationally. Overall, the function and pathways of CDCAs and their 50 frequently altered neighboring genes showed that these genes were mainly enriched in changes in cell division, mitotic nuclear division, protein binding and other cell functions. KEGG pathway analysis showed an enrichment in PI3K-Akt and AMPK signaling pathway, as well as some cancers and cancer-related signaling pathway. Thus, modifications to CDCAs is associated with post-transcriptional regulation, which is largely linked to protein translation.

To date, no studies have investigated the role of CDCAs and the connection between tumor infiltrating immune cells and HNSCC. We first explored the difference between cumulative survival between HNSCC, HNSCC-HPV-pos and HNSCC-HPV-neg tumors and found that HNSCC-HPV-pos group had a significantly higher infiltration of B cells, CD8+ T cells and neutrophil cells (P<0.05), which was positively related to CDCAs expression. This indicates that immune cells may have a significant effect on the prognosis of this disease. Therefore, it is worth further investigation in subsequent studies. There were several limitations, one being that all the data in our study was based on online free databases. Additionally, our study does not provide precise clinical information. Hence, more studies are needed to prove our findings. Another limitation is that we did not assess the possible therapeutic and diagnostic roles of CDCAs as the histological types of HNSCC as well as the multiple anatomical sites of the cancer varies widely. Thus, future studies are needed. Finally, we were incapable to contrast the differences in function of CDCAs among HPV-positive and HPV-negative in HNSCC due to insufficient data, though we plan to investigate this in the future.

Conclusion

Our results indicate that CDCAs play a key role in the HPV-pos HNSCC patients. This study made use of online free tools to perform target gene analyses on HNSCC from open databases, which enables more genomics research and subsequent functional exploration.

Supporting information

S1 Appendix. Functions and pathways of CDCAs and their 50 frequently altered neighboring genes were analyzed by GO and KEGG in DAVID online database. (DAVID database). (XLSX)

Author Contributions

Project administration: Ming Fang.
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References

1. Xiao-Nan F, Miao Y, Hua L. Comprehensive analysis of competitive endogenous RNAs network associated with head and neck squamous cell carcinoma. Scientific Reports, 2018, 8(1):10544-. https://doi.org/10.1038/s41598-018-28957-y PMID: 3002503

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6):394–424. https://doi.org/10.3322/caac.21492 PMID: 30207593

3. Magnes T, Egie A, Greil R, Melchart T. Update on squamous cell carcinoma of the head and neck: ASCO annual meeting 2017. Memo. 2017; 10(4):220–223. https://doi.org/10.1007/s12254-017-0358-9 PMID: 29250200

4. Chin D, Boyle GM, Williams RM, Ferguson K, Pandeya N, Pedley Jet al. Coman WB: Novel markers for poor prognosis in head and neck cancer. Int J Cancer 2005, 113:789–797. https://doi.org/10.1002/ijc.20608 PMID: 15499618

5. Bressan V, Stevanin S, Bianchi M, Aleo G, Bagnasco A, Sasso L. The effects of swallowing disorders, dysgeusia, oral mucositis and xerostomia on nutritional status, oral intake and weight loss in head and neck cancer patients: a systematic review. Cancer Treat Rev. 2016; 45:105–119. https://doi.org/10.1016/j.ctrv.2016.03.006 PMID: 27010487

6. Coskun HH, Medina JE, Robbins KT. Current philosophy in the surgical management of neck metastases for head and neck squamous cell carcinoma. Head Neck. 2015; 37(6):915–926. https://doi.org/10.1002/hed.23689 PMID: 24623715

7. Ni G, Huang K, Luan Y. Human papillomavirus infection among head and neck squamous cell carcinomas in southern China. PLoS One. 2019 Sep 23; 14(9):e0221045. https://doi.org/10.1371/journal.pone.0221045 PMID: 31545422

8. Zhao M, Hu X, Xu Y. Targeting of EZH2 inhibits epithelial-mesenchymal transition in head and neck squamous cell carcinoma via regulating the STAT3/VEGFR2 axis. Int J Oncol. 2019; 55(5):1165–1175. https://doi.org/10.3892/ijo.2019.4880 PMID: 31545422

9. Valentl F, Sacconi A, Ganci F. The miR-205-5p/BRCA1/RAD17 Axis Promotes Genomic Instability in Head and Neck Squamous Cell Carcinomas. Cancers (Basel). 2019 Sep 11; 11(9).

10. Wang L, Jia Y, Jiang Z, Gao W, Wang B. FSCN1 is upregulated by SNAI2 and promotes epithelial to mesenchymal transition in head and neck squamous cell carcinoma. Cell Biol Int. 2017; 41(8):833–841. https://doi.org/10.1002/cbi.10786 PMID: 2848774

11. Phan N N, Wang C Y, Li K L, Chen C F, Chiao C C, Yu H G et al. Distinct expression of CDCA3, CDCA5, and CDCA8 leads to shorter relapse free survival in breast cancer patient. Oncotarget, 2018, 9(6):6977–6992. https://doi.org/10.18632/oncotarget.24059 PMID: 29467944

12. Obara W, Sato F, Takeda K. Phase I clinical trial of cell division associated 1 (CDCA1) peptide vaccination for castration resistant prostate cancer. Cancer Science, 2017, 108(7):1452–1457. https://doi.org/10.1111/cas.13278 PMID: 28498618

13. Thang P, Takano A, Yoshitake Y. Cell division cycle associated 1 as a novel prognostic biomarker and therapeutic target for oral cancer. International Journal of Oncology, 2016, 49(4):1385. https://doi.org/10.3892/ijo.2016.3800

14. Walker MG. Drug target discovery by gene expression analysis cell cycle genes. Curr Cancer Drug Targets, 2001, 1(1):73–83. https://doi.org/10.2174/1568009013334241 PMID: 12188893

15. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia. 2007: 9:166–80. https://doi.org/10.1593/neo.07112 PMID: 17356713

16. Asplund A, Edqvist PH, Schwenk JM, Pontén F. Antibodies for profiling the human proteome-The Human Protein Atlas as a resource for cancer research. Proteomics, 2012; 12:20067–77. https://doi.org/10.1002/pmic.201100504 PMID: 22623277

17. Tang Z.; Li C.; Kang B.; Gao G.; Li C.; Zhang Z. Gepia: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017, 45, W98–W102. https://doi.org/10.1093/nar/gkx247 PMID: 28407145

18. ChandraShekar DS, Baskell B, Balasubramanya SAH, Creighton CJ, Rodriguez IP, Chakravarthi BVSKet al. UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia. 2017 Aug; 19(8):649–658. https://doi.org/10.1016/j.neo.2017.05.002 PMID: 28732212
19. Nagy A, Lánzczky A, Menyhárt O, Győrffy B. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets, Scientific Reports, 2018; 8:9227. https://doi.org/10.1038/s41598-018-2752-y PMID: 29907753

20. Cerami E, Gao J, Dogrusoz U. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data [published correction appears in Cancer Discov. 2012 Oct;2(10):960]. Cancer Discov. 2012; 2(5):401–404. https://doi.org/10.1158/2159-8290.CD-12-0095 PMID: 22588877

21. Li T, Fan J, Wang B. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Research, 2017, 77(21):e108–e110. https://doi.org/10.1158/0008-5472.CAN-17-0307 PMID: 29092952

22. Peng CH, Liao CT, Peng SC. A novel molecular signature identified by systems genetics approach predicts prognosis in oral squamous cell carcinoma. PLoS One. 2011; 6(8):e23452. https://doi.org/10.1371/journal.pone.0023452 PMID: 21853135

23. Dohun P, Newton M A, Lambert P F. Fundamental differences in cell cycle deregulation in human papillomavirus-positive and human papillomavirus-negative head/neck and cervical cancers. Cancer Research, 2007, 67(10):4605–4619. https://doi.org/10.1158/0008-5472.CAN-06-3619 PMID: 17510386

24. Sengupta S, Ih D B J, Newton M A. Genome-Wide Expression Profiling Reveals EBV-Associated Inhibition of MHC Class I Expression in Nasopharyngeal Carcinoma. Cancer Research, 2006, 66(16):7999–8006. https://doi.org/10.1158/0008-5472.CAN-05-4399 PMID: 16912175

25. Ye H, Yu T, Temam S. Transcriptomic dissection of tongue squamous cell carcinoma[J]. BMC Genomics, 2008, 9(1):69–0.

26. Giordano T. J. Delineation, Functional Validation, and Bioinformatic Evaluation of Gene Expression in Thyroid Follicular Carcinomas with the PAX8-PPARG Translocation. Clinical Cancer Research, 2006, 12(7):1983–1993. https://doi.org/10.1158/1078-0432.CCR-05-2039 PMID: 16609007

27. Tokuzumi A, Fukushima S, Miyashita A. Cell division cycle-associated protein 1 as a new melanoma-associated antigen. J Dermatol. 2016; 43(12):1399–1405. https://doi.org/10.1111/1346-8138.13436 PMID: 27237743

28. Tomita Y, Yuno A, Tsukamoto H. Identification of CDCA1-derived long peptides bearing both CD4 + and CD8 + T-cell epitopes: CDCA1-specific CD4 + T-cell immunity in cancer patients[J]. International Journal of Cancer, 2013, 134(2):352–366.

29. Kaneko N, Miura K, Gu Z. siRNA-mediated knockdown against CDCA1 and KNTC2 both frequently overexpressed in colorectal and gastric cancers suppresses cell proliferation and induces apoptosis. Biochem Biophys Res Commun. 2009, 390 (4): 1235–1240. https://doi.org/10.1016/j.bbrc.2009.10.127 PMID: 19878654

30. Vagnarelli P. Repo-man at the intersection of chromatin remodeling, DNA repair, nuclear envelope organization and cancer progression. Adv Exp Med Biol. 2014, 773: 401–414. https://doi.org/10.1007/978-1-4899-8032-8_18 PMID: 24963358

31. Qian J, Lesage B, Beullens M, Van Eynde A, Bollen M. PP1/repo-man dephosphorylates mitotic histone H3 at T3 and regulates chromosomal aurora B targeting. Curr Biol. 2011; 21(9):766–73. https://doi.org/10.1016/j.cub.2011.03.047 PMID: 21514157

32. Krasnoselsky AL, Whiteford CC, Wei JS, Bilke S. Altered expression of cell cycle genes distinguishes aggressive neuroblastoma. Oncogene. 2005; 24(9):1533–41. https://doi.org/10.1038/sj.onc.1208341 PMID: 15592497

33. Uchida F, Uzawa K, Kasamatsu A. Overexpression of CDCA2 in human squamous cell carcinoma: correlation with prevention of G1 phase arrest and apoptosis. PLoS One. 2013; 8(2):e56381. https://doi.org/10.1371/journal.pone.0056381 PMID: 23418564

34. Run S, Chun R, Ya Q. CDC2A2 promotes lung adenocarcinoma cell proliferation and predicts poor survival in lung adenocarcinoma patients. Oncotarget. 2017; 8(12):19768–79. https://doi.org/10.18632/oncotarget.15519 PMID: 28423619

35. Feng Y, Qian W, Zhang Y. CDC2A2 promotes the proliferation of colorectal cancer cells by activating the AKT/CCND1 pathway in vitro and in vivo. BMC Cancer. 2019 Jun 13; 19(1):576. https://doi.org/10.1186/s12885-019-5793-z PMID: 31196027

36. Zhang W, Lu Y, Li X. CDC2A3 promotes cell proliferation by activating the NF-kappaB/cyclin D1 signaling pathway in colorectal cancer. Biochem Biophys Res Commun, 2018, 500(2): 196–203. https://doi.org/10.1016/j.bbrc.2018.04.034 PMID: 29627567

37. Yu J, Hua R, Zhan Y, Tao R, Wang Q, Ni Q. DNA hypomethylation promotes invasion and metastasis of gastric cancer cells by regulating the binding of SP1 to the CDC2A3 promoter. J Cell Biochem. 2020; 121(1):142–151. https://doi.org/10.1002/jcb.28993 PMID: 31211445
38. Zhang Y, Yin W, Cao W. CDCA3 is a potential prognostic marker that promotes cell proliferation in gastric cancer. Oncol Rep. 2019 Apr; 41(4):2471–2481. https://doi.org/10.3892/or.2019.7008 PMID: 30816466

39. Chen J, Zhu S, Jiang N. HoxB3 promotes prostate cancer cell progression by transactivating CDCA3 [J]. Cancer Letters, 2013, 330(2):217–224. https://doi.org/10.1016/j.canlet.2012.11.051 PMID: 23219899

40. Abdullah J M, Jing X, Spassov D S. Cloning and Characterization of Hepp, a Novel Gene Expressed Preferentially in Hematopoietic Progenitors and Mature Blood Cells. Blood Cells Molecules & Diseases, 2001, 27(3):0–676.

41. Xu Y, Wu X, Li F, Huang D, Zhu W. CDCA4, a downstream gene of the Nrf2 signaling pathway, regulates cell proliferation and apoptosis in the MCF-7/ADM human breast cancer cell line. Mol Med Rep. 2018; 17(1):1507–1512. https://doi.org/10.3892/mmr.2017.8095 PMID: 29257222

42. Pang S, Xu Y, Chen J. Knockdown of cell division cycle-associated protein 4 expression inhibits proliferation of triple negative breast cancer MDA-MB-231 cells in vitro and in vivo. Oncol Lett. 2019 May; 17(5):4393–4400. https://doi.org/10.3892/ol.2019.10077 PMID: 30944632

43. Chang IW, Lin VC, He HL. CDC5 overexpression is an indicator of poor prognosis in patients with urothelial carcinomas of the upper urinary tract and urinary bladder. Am J Transl Res, 2015, 7(4):710–722. PMID: 26064439

44. Xu J, Zhu C, Yu Y. Systematic cancer-testis gene expression analysis identified CDC5 as a potential therapeutic target in esophageal squamous cell carcinoma. EBioMedicine. 2019 Aug; 46:54–65. https://doi.org/10.1016/j.ebiom.2019.07.030 PMID: 31324603

45. Chen H, Chen J, Zhao L. CDC55, Transcribed by E2F1, Promotes Oncogenesis by Enhancing Cell Proliferation and Inhibiting Apoptosis via the AKT Pathway in Hepatocellular Carcinoma. J Cancer. 2019 Apr 21; 10(8):1846–1854. https://doi.org/10.7150/jca.28809 PMID: 31055411

46. Clermont P L, Crea F, Jiang W T. Identification of the epigenetic reader CBX2 as a potential drug target in advanced prostate cancer[J]. Clinical Epigenetics, 2016, 8(1):16.

47. Clermont P L, Sun L, Crea F. Genotranscriptomic meta-analysis of the Polycomb gene CBX2 in human cancers: initial evidence of an oncogenic role[J]. British Journal of Cancer, 2014, 111(8):1663–1672.

48. Jimenez PR, Martin-Cortazar C, Kourani O. CDC6 is a critical mediator of lymphomagenesis that selectively regulates anchorage-independent growth. Haematologica, 2018, 103(10):1669–1678.

49. Ye L, Li F, Song Y. Overexpression of CDC7 predicts poor prognosis and induces EZH2-mediated progression of triple-negative breast cancer. Int J Cancer. 2018 Nov 15; 143(10):2602–2613. https://doi.org/10.1002/ijc.31766 PMID: 30151890

50. Jenness C, Giunta S, Manuel M, Müller. HELLS and CDC7 comprise a bipartite nucleosome remodeling complex defective in ICF syndrome[J]. Proceedings of the National Academy of Sciences, 2018, 115(5):201717509

51. Thijssen P E, Ito Y, Grillo G. Mutations in CDC7 and HELLS cause immunodeficiency–centromeric instability–facial anomalies syndrome[J]. Nature Communications, 2015, 6:7870. https://doi.org/10.1038/ncomms8870 PMID: 26216346

52. Gill R M, Gabor T V, Couzens A L. The MYC-Associated Protein CDC7 is Phosphorylated by AKT To Regulate MYC-Dependent Apoptosis and Transformation[J]. Molecular and Cellular Biology, 2013, 33 (3):498–513. https://doi.org/10.1128/MCB.00276-12 PMID: 23166294

53. Hayama S, Daigo Y, Yamabuki T. Phosphorylation and Activation of cell division cycle associated 8 by aurora kinase B plays a significant role in human lung carcinogenesis. Cancer Res, 2007, 67(9):4113–4122. https://doi.org/10.1158/0008-5472.CAN-06-4705 PMID: 17483322

54. Ci C, Tang B, Lu D. Overexpression of CDC8B promotes the malignant progression of cutaneous melanoma and leads to poor prognosis. Int J Mol Med. 2019 Jan; 43(1):404–412. https://doi.org/10.3892/ijmm.2018.3985 PMID: 30431060

55. Bu Y, Shi L, Yu D. CDC8A is a key mediator of estrogen-stimulated cell proliferation in breast cancer cells. Gene. 2019 Jun 30; 703:1–6. https://doi.org/10.1016/j.gene.2019.04.006 PMID: 30953709

56. Wang Y, Zhao Z, Bao X. Borealin/Dasar B is overexpressed in colorectal cancers and contributes to proliferation of cancer cells[J]. Medical Oncology, 2014, 31(11):248. https://doi.org/10.1007/s12032-014-0248-5 PMID: 25260804

57. Hayama S, Daigo Y, Yamabuki T. Phosphorylation and Activation of Cell Division Cycle Associated 8 by Aurora Kinase B Plays a Significant Role in Human Lung Carcinogenesis. Cancer Research, 2007, 67(9):4113–4122.