Abstract

Acquired uniparental disomy (aUPD) leads to homozygosity facilitating identification of monoallelically expressed genes. We analyzed single-nucleotide polymorphism array-based genotyping data of 448 head and neck squamous cell carcinoma (HNSCC) samples from The Cancer Genome Atlas to determine the frequency and distribution of aUPD regions and their association with survival, as well as to gain a better understanding of their influence on the tumor genome. We used expression data from the same dataset to identify differentially expressed genes between groups with and without aUPD. Univariate and multivariable Cox proportional hazards models were performed for survival analysis. We found that 82.14% of HNSCC samples carried aUPD; the most common regions were in chromosome 17p (31.25%), 9p (30.13%), and 9q (27.46%). In univariate analysis, five independent aUPD regions at chromosome 9p, two regions at chromosome 9q, and the CDKN2A region were associated with poor overall survival in all groups, including training and test sets and human papillomavirus (HPV) negative samples. Forty-three genes in areas of aUPD including PDL1 and CDKN2A were differentially expressed in samples with aUPD compared to samples without aUPD. In multivariable analysis, aUPD at the CDKN2A region was a significant predictor of overall survival in the whole cohort and in patients with HPV negative HNSCC. aUPD at specific regions in the genome influences clinical outcomes of HNSCC and may be beneficial for selection of personalized therapy to prolong survival in patients with this disease.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer worldwide; more than half a million new patients are diagnosed each year [1]. Incidence has increased, especially among young patients, because of increasing prevalence of human papillomavirus (HPV) [2,3]. The 5-year overall survival (OS) rate is better in patients with HPV-associated HNSCC than in those whose tumors are not associated with HPV [4].

Loss of heterozygosity (LOH) results from loss of one of two parental alleles present in each genome. In most cases LOH results in cells having a single copy of one parental allele and loss of the other allele. Acquired uniparental disomy (aUPD) also called copyneutral LOH is a subset of LOH wherein a chromosomal region or whole chromosome is lost and reduplicated. aUPD is not associated with changes in copy number. Thus each cell harbors two copies of a single parental allele rather than one copy each of two parental alleles. Both regulatory and open reading frames are monoallelic and any alterations in promoter, enhancer or regions either as the result of germline SNPs or methylation that are included in the aUPD could alter the expression or stability of mRNAs or the stability of function of their protein products. aUPD thus has the potential to expose effects of homozygosity for existing germline and somatic aberrations including mutations, deletions, methylation (hypo or hyper), complex structural alterations, and imprinted genes [5–10]. aUPD can be a consequence of mitotic recombination that usually results in segmental aUPD where only a portion of the chromosome arises from a single parent. Whole chromosome aUPD is usually the consequence of anaphase lagging of one chromosome, and with duplication of the whole chromosome [11–15]. Moreover, loss of chromosomal segments or whole chromosome, and frequently duplication of the retained allele in subsequent replication can be consequence of breakage fusion bridge (BFB) events in cancer [16]. Thus, a UPD can be a result of BFB and thereafter replication of the retained allele [16]. Genomic copy number alterations, gene expression, miRNA expression, and protein expression are well studied in HNSCC. Accumulating data have shown that genomic events including loss of heterozygosity (LOH) and epigenetic changes pre-

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sent in tumors can be used as prognostic biomarkers for cancer [17–19]. However, genomewide profiling of aUPD in HNSCC is very limited with most studies having small sample sizes [20–22]. Of note, aUPD profiling and association between aUPD regions and survival have been reported in a variety of malignancies including MDS, MDS/MPD, and secondary AML [23]. However, allelabeled changes in the genome and their association with clinical outcome and survival are poorly characterized, and acquired uniparental disomy (aUPD) has not been studied as a potential prognostic factor in HNSCC.

Previously, we have shown that smallest overlapping regions of aUPD were associated with etiologic factors such as alcohol intake, smoking, HPV and TP53 mutation status of HNSCCs [24]. In the current study, we profiled genomewide aUPD to determine the frequency and distribution of aUPD in HNSCC and to determine whether any smallest overlapping regions (SORs) of aUPD were associated with survival and clinical characteristics of disease in a large data set. Importantly, we assessed expression of mRNA for genes located in areas of aUPD to determine effects of monoallelic gene expression due to aUPD on gene expression. This represents the first large scale comprehensive study of aUPD regions and their association with survival in HNSCC.

Materials and Methods

Patient Samples

Clinical and patient demographic data were retrieved from TCGA (http://portal.gdc.cancer.gov/). We noted HPV status as reported by TCGA and by Nulton et al. [25,26]. Patient characteristics are summarized in Table S1a, and all samples are listed in Table S1B. Overall survival (OS) was calculated from the date of diagnosis of head and neck cancer to the date of death or last followup. Recurrencefree survival (RFS) was calculated from the date of diagnosis of HNSCC to the date of recurrence or last followup. Sample and clinical data were based on a November 2015 freeze from TCGA data portal.

Genomic Data and aUPD Analysis

Genomic data (CEL files) were retrieved from TCGA data portal. Genotyping console software (Affymetrix) was used to perform quality control, and then to generate CHP files. Affymetrix contrast QC threshold was used for both tumor and matching samples. Data that did not pass the quality control check were removed from further analysis; 448 samples (448 tumor and 448 matching normal) passed the quality control check and were used in the study. aUPD analysis and detection of SORs of aUPD were performed using Copy Number Analyzer for GeneChip (CNAG v4.0) software (http://www.genome.umin.jp) by using tumor and matching normal data as described previously [10,27]. The SORs of UPD are described based on the 3 and 5 endpoints of aUPD regions. The Dec 2013 human genome browser (NCBI Build 38/hg38; http://genome.ucsc.edu) was used for identification of gene localization. Telomeric aUPD was defined as aUPD occurring in the telomeric region with one breakpoint. When at least two breakpoints appeared, it was defined as centromeric aUPD (also called interstitial). Segmental aUPD was defined as all centromeric and telomeric regions. When aUPD occurred in whole arm or whole arm of chromosome, it was defined as whole arm or whole arm aUPD. If aUPD occurred in the whole chromosome, it was considered wholechromosome aUPD (Figure S1A). Total aUPD represented both segmental and wholechromosome aUPD. We recruited normalized HiSeq gene expression data from Cancer Genome Browser (https://xena.ucsc.edu/) to determine differentially expressed genes between samples with and without aUPD.

Statistical Analysis

The nonparametric KruskalWallis test was used to analyze the correlation between aUPD (total, telomeric, centromeric, segmental, wholechromosome, whole arm, and whole arm) and clinical characteristics (stage, grade, HPV status, and gender). Univariate Cox proportional hazards models were used to determine the effects of SORs of aUPD, gender, age, stage, and grade on OS and RFS. KaplanMeier analysis and logrank p values were calculated to identify survival differences between groups. A multivariable Cox proportional hazards model was used to select prognostic markers. This study complied with REMARK criteria [28]. A two-tailed Student t test was used to compare expression of genes between samples with aUPD and those without aUPD for identified SORs. To correct for multiple comparisons, we adjusted the p values by obtaining the BenjaminiHochberg false discovery rate [29]. A finding was considered significant when the twosided P value was less than .05. Statistical analyses were performed in STATA v10 (STATA Corp., College Station, TX).

Results

Profiling SORs of aUPD

We analyzed single nucleotide polymorphism based arrays generated by The Cancer Genome Atlas (TCGA) from 448 HNSCCs to identify the frequency and distribution of segmental and wholechromosome aUPD. A total of 1591 aUPD regions were found across all of the chromosomes (per sample range 0 to 27, mean 3.56, median 3.0), including 1407 segmental and 184 wholechromosome events in the entire data set. The frequency of HNSCC samples harboring at least one aUPD was 82.14% (368 of the 448 samples). The most common instances of aUPD were found in chromosomes 17p (31.25%), 9p (30.13%), 9q (27.46%), and 13q (18.53%) (Figure S1B).

Next we tested for differences in the frequency of total, telomeric, centromeric, segmental region, wholechromosome, whole arm, and whole arm aUPD among patient samples by disease stage, grade, and HPV status. In KruskalWallis tests, the frequency of total, telomeric, centromeric, segmental aUPD was significantly higher in patients with stage III and IV disease than in those with stage I and II disease (total: P = .005, telomeric: P = .011, centromeric: P = .021, segmental: P = .002), but no differences were found by disease stage in whole arm (P = .278), whole arm (P = .375), and whole chromosome (P = .4821; Figure 1). In contrast to disease stage, the frequency of aUPD was not correlated with grade (total: P = .733, telomeric: P = .793, centromeric: P = .133, segmental: P = .826, wholechromosome: P = .819, whole arm: P = .675, whole arm: P = .352; Figure S2). The frequencies of total (P = .036) aUPD were significantly higher in HPVnegative patients than in HPVpositive patients. However, no differences were found according to HPV status for telomeric (P = .472), centromeric (P = .962), segmental (P = .102), wholechromosome (P = .188), or whole arm (P = .810), and whole arm (P = .389) aUPD (Figure S3).

Association of Recurrent SORs of aUPD With Survival

We identified 23 SORs of aUPD, including CDKN2A, across all chromosomes. Independent SORs were identified at chromosomes 2q (two regions), 6p (one region), 9p (10 regions), 9q (four regions), 11q (two regions), 13q (one region), and 17p (two regions), as well as CDKN2A (at chromosome 9p) (see Table 1 and Supplemental Table S2). Next, we tested whether any of these SORs were associated with OS or recurrencefree survival (RFS). First we randomly divided samples into a training and a test sets (Table S1) and assessed whether the SORs were associated with survival. In univariate analysis, SORs of aUPD at chromo-
some 9p (9p24.3; \(P = .044\), 9p24.1; \(P = .020\), 9p23p22.3; \(P = .009\), 9p22.3p22.2; \(P = .014\), 9p21.3_1; \(P = .017\), 9p21.3_2; \(P = .024\), 9p21.3p21.2; \(P = .044\), 9p21.2; \(P = .008\), 9p21.1; \(P = .044\), and 9p13.3; \(P = .029\), respectively; Table 1, Figs. 2 and 3, Table S2a, and Figure S4) and CDKN2A (\(P = .045\); Table 1, Figure 3), as well as gender (\(P = .022\); Table 1, Figure S4), were associated with shorter OS in the training set. In the test set, one SOR of anUPD at chromosome 6p12.3 (\(P = .018\)), seven SORs at chromosome 9p (9p24.3; \(P = .008\), 9p24.1; \(P = .007\), 9p23p22.3; \(P = .029\), 9p22.3p22.2; \(P = .011\), 9p21.3_1; \(P = .010\), 9p21.3_2; \(P = .018\), and 9p21.3p21.2; \(P = .033\), respectively), two SORs at 9q (9q33.2; \(P = .025\) and 9q34.13; \(P = .026\)), and one SOR at the CDKN2A (9p21.3; \(P = .008\)) were associated with reduced OS (Table 1, Figures 2 and 3, Table S2B, Figures S4 and S5). In the test set, only two SORs of anUPD at 11q (11q22.3; \(P = .008\); and 11q25;...
were associated with reduced RFS (Table S2B). In the training set, none of the SORs of aUPD were associated with RFS (Table S2a). In multivariable analysis, 9p21.2 ($P < .0001$) in training set, and 9p24.1 ($P < .0001$) and 9p23p22.3 ($P < .0001$) in test set were associated with worst OS, while none of SOR of aUPD was a significant predictor of RFS in training and test sets (Table 2).

Then we tested all samples to determine whether the SORs were associated with survival. In univariate analysis, aUPD in 14 independent SORs was associated with reduced OS in all samples; 10 regions at chromosomes 9p (9p24.3; $P = .001$, 9p24.1; $P < .0001$, 9p23p22.3; $P = .001$, 9p22.3p22.2; $P = .001$, 9p21.3_1; $P < .0001$, 9p21.3_2; $P = .001$, 9p21.3p21.2; $P = .002$, 9p21.2; $P = .002$, 9p21.1; $P = .027$, and 9p13.3; $P = .004$) and four at chromosome 9q (9q22.33; $P = .025$, 9q31.3; $P = .007$, 9q33.2; $P = .006$, and 9q34.13; $P = .012$; Table 1, Table S2C, Figures 2 and 3, Figures S4 and S5). Conversely, only two SORs of aUPD at chromosome 11q (11q22.3; $P = .006$, and 11q25; $P = .006$) were associated with reduced RFS. In addition, the SOR of aUPD at CDKN2A ($P = .001$; Figure 3) was associated with shorter OS, but deletion at the same region was not associated with OS ($P = .235$). Gender ($P = .009$) was also associated with reduced OS. In multivariate analysis, SORs of aUPD at chromosome 9p23p22.3 ($P = .001$, 9p21.3_1; $P < .0001$, 9p21.3_2; $P = .001$, 9p21.3p21.2; $P = .002$, 9p21.2; $P = .001$, 9p21.1; $P = .024$, and 9p13.3; $P = .009$) and chromosome 9q (9q22.33; $P = .014$, 9q31.3; $P = .003$, 9q33.2; $P = .005$, and 9q34.13; $P = .007$; Table 1, Figures 2 and 3, Table S2D, Figure S4). Similar to all samples, only two SORs at chromosome 11q (11q22.3; $P = .006$, and 11q25; $P = .006$) were associated with shorter RFS. In contrast to all samples, and HPV negative samples, none of the SORs were associated with OS or RFS in HPV positive samples.

**Association of Differentially Expressed Genes in the SORs of aUPD with Survival**

Seventeen of 22 SORs in all samples, including the CDKN2A, and additional one region in the test set, were associated with survival. The 18 SORs contained 135 genes (Table S3A). The number of genes in these
18 SORs varied. Two SORs at chromosome 9p (9p21.3_2 and 9p21.1) did not contain any open reading frames (ORF), however both contain long noncoding RNA and pseudogenes, and 9p21.3_2 also harbors regulatory elements (Supplementary Table S3B). One of independent SOR at 6p12.3, four at chromosome 9p (9p24.3, 9p23-p22.3, 9p21.3_2, and 9p21.3p21.2, one at 9q31.3, and one at 11q25 contained only one protein-coding gene; the remaining SORs consisted of multiple genes (e.g., 9p13.3 had 51 genes; Table S3A). Moreover, miRNAs or and noncoding RNAs as well as pseudogenes and regulatory elements (promoter or enhancers) are mapped in the 18 regions explored in this manuscript and may contribute to the correlations with outcomes (Supplementary Table S3B).

Next, we tested whether the expression of these 135 genes differed between samples with and without aUPD. Fiftysix genes were significantly differentially expressed between samples with and without aUPD. With Benjamini-Hochberg false discovery rate correction, only 43 genes were significantly differentially expressed. Fortyone of these 43 genes had significantly higher expression in aUPD-positive samples than in aUPD-negative samples. Significantly overexpressed genes included CD274 (also known as PDL1; \( q = 8.99 \times 10^{-6} \)) in SOR at chromosome 9p24.1, and DCTN3 and VCP (\( q = 2.86 \times 10^{-7} \)) at chromosome 9p13.3. We found that only two of the 43 genes had significantly lower expression in aUPD-positive samples than in aUPD-negative samples: CDKN2A at chromosome 9p21.3 and GGTA1 at chromosome 9q33.2 (\( P = 3.78 \times 10^{-4} \); Table S3).

**Discussion**

Our results indicate that aUPD is widespread in HNSCC, and specific SORs of aUPD in the genome influence survival. We also found that

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**Table 2. Multivariable analysis of clinical and genetic covariates for OS and RFS in patients with head and neck squamous cell carcinoma**

| Variable | HR (95% CI) | \( P \)  |
|----------|-------------|--------|
| OS       |             |        |
| Training set | 2.95E+09 (8.67E+07-1.01E+11) | <.0001 |
| Test set  | 4.54E+07 (5.88E+06-3.50E+08) | <.0001 |
| All samples | 5.06E-08 (1.95E-09-1.32E-06) | <.0001 |
| 9p21.3_1 | 61.34 (1.59E+09-2.36E+09) | .027 |
| 9p21.2   | 7.36E-08 (1.09E-08-5.67E-09) | <.0001 |
| 9p21.1   | 0.26 (0.09-0.79) | .017 |
| CDKN2A   | 0.13 (0.02-0.85) | .034 |
| HPV-negative samples | 7.85E-10 (1.05E-10-5.88E-09) | <.0001 |
| 9p21.3-p21.2 | 0.29 (0.09-0.88) | .029 |
| CDKN2A   | 0.13 (0.02-0.92) | .041 |
| RFS All samples | 8.63 (1.18E+06-6.31E+05) | .034 |
| 11q23   | 3.66 (1.45-9.23) | .006 |

Abbreviations: OS, overall survival; RFS, recurrence free survival; HR, hazard ratio; CI, confidence interval; \( q \), Benjamini-Hochberg false discovery rate.

Only variables that were significant in univariate analysis were included in multivariate analysis. \* \( P < .05 \) was used to select features.

18 SORs varied. Two SORs at chromosome 9p (9p21.3_2 and 9p21.1) did not contain any open reading frames (ORF), however both contain long noncoding RNA and pseudogenes, and 9p21.3_2 also harbors regulatory elements (Supplementary Table S3B). One of independent SOR at 6p12.3, four at chromosome 9p (9p24.3, 9p23-p22.3, 9p21.3_2, and 9p21.3p21.2, one at 9q31.3, and one at 11q25 contained only one protein-coding gene; the remaining SORs consisted of multiple genes (e.g., 9p13.3 had 51 genes; Table S3A). Moreover, miRNAs or and noncoding RNAs as well as pseudogenes and regulatory elements (promoter or enhancers) are mapped in the 18 regions explored in this manuscript and may contribute to the correlations with outcomes (Supplementary Table S3B).

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**Discussion**

Our results indicate that aUPD is widespread in HNSCC, and specific SORs of aUPD in the genome influence survival. We also found that
genes within these SORs were differentially expressed between those with and without aUPD.

The mechanisms influencing cancer development may vary among the telomeric, centromeric, whole arm and whole chromosomes aUPD. Thus, in the current study, we analyzed the data for the wholep and wholeq arm, whole chromosome, telomeric and centromeric aUPD. We found that the frequency of total aUPD, as well as telomeric and centromeric was associated with stage. However, the frequency of aUPD was not associated with grade. These findings contrast with those of our previous report in highgrade serous epithelial ovarian cancers, in which the frequency of aUPD was associated with grade but not with stage [10]. This suggests that aUPD can mediate different functions across cancer lineage.

We identified a total of 18 SORs of aUPD that were associated with shorter OS or RFS. Recently aUPD has been shown to occur at areas encompassing imprinted genes in tumors [8]. The 18 SORs encompass 135 genes and the expression of 43 of the 135 genes within the defined aUPD regions was significantly different compared with samples without aUPD in the same regions. Thus the monoallelic expression of the 43 differentially expressed genes may alter expression. Differentially expression of the 43 genes identified to be located in aUPD SOR in the current study may confer an advantage for the tumor, and may contribute to tumor aggressiveness. Fortyone of the 43 differentially expressed genes had significantly higher expression and only two had lower expression in samples with aUPD compared with those without aUPD. If these genes are in areas encompassing imprinting [8], only the nonimprinted gene was selected. This finding indicates that many of these genes may exhibit gain of function activity. PDL1 hypomethylation has been observed in HNSCC in TCGA and other data sets. PDL1 hypomethylation is inversely correlated with mRNA and protein expression [25,30]. If a hypomethylated PDL1 allele undergoes reduction to homozygosity through aUPD, this could result in increased PDL1 mRNA expression as is observed in samples with aUPD compared to those without aUPD.

Six SORs of aUPD at chromosome 9p (9p22.3p22.2, 9p21.3_1, 9p21.3_2, 9p21.3p21.2, and the C9orf23), were associated with shorter OS in all groups, including the training and test sets, all samples, and HPVnegative samples. Moreover, seven SORs at chromosome 9p (9p24.3, 9p24.1, 9p22.3p22.2, 9p21.3_1, 9p21.3_2, 9p21.3p21.2) and two SORs at chromosome 9q (9q33.2 and 9q34.13) were associated with reduced OS in the test set, all samples, and HPVnegative samples. In contrast, only two SORs of aUPD at chromosome 11q (11q22.3 and 11q25) were associated with RFS in the test set, all samples, and HPVnegative samples. This may be partly because data for RFS in some samples were missing decreasing the power to detect associations. Although only a few samples had SORs of aUPD at chromosome 11q (11q22.3 and 11q25), the overall concordance of the data among the training set, test set, all samples, and HPVnegative samples was notable.

Previously aUPD (in 3 out16 samples) and deletion (in 13 out16 samples) at chromosome 9p were reported in HNSCC [22], and at chromosome 3p and 17 [20]. However these regions were not refined and association between aUPD regions and survival was not assessed due to the small sample sets. LOH at 8p21.2 and 9p21.2 has been reported to be associated with shorter survival in HNSCC samples [17]. LOH at 9p was also found to be predictive for local relapse in HNSCC [19]. However, in both LOH studies microsatellite markers were employed to identify LOH in limited regions in HNSCC samples and the LOH was not further segregated into copy number loss and aUPD [17,19]. Genomewide LOH analysis was performed in the TCGA HNSCC paper based on deletion, but LOH or aUPD were not analyzed for association with gene expression or survival in the TCGA HNSCC paper [25].

CD274 (also known as PDL1), RCLI, PDCD11G2 (also known as PDL2), KIAA1432, JAK2 at chromosome 9p24.1, and VCP, DCTN3, STOML2, 9orf23, and GALT at chromosome 9p13.3, and CDKN2A at chromosome 9p21.3 and TTF1 at chromosome 9q34.13 provide examples of significantly differentially expressed genes between samples with and without aUPD that were associated with OS in all samples, HPVnegative samples, and the test set. CD274 (also known as PDL1) encodes programmed cell death protein1 ligand 1, which is an immune inhibitory receptor ligand, and interaction of this ligand with its receptor, PD1 (programmed cell death protein1), inhibits Tcell activation and cytokine production and enables immune escape. PDL1 is primarily expressed in cancer cells, parenchymal cells, and myeloid cells, whereas PD1 is primarily expressed in tumorinfiltrating lymphocytes. Activation of the PD1/ PDL1 axis occurs in tumors either through innate immune resistance or adaptive immune resistance [31]. Previously, overexpression of PDL1 has been shown in a variety of cancers, including HNSCC [32], gastric cancer [33], cervical cancer [34], and squamous carcinoma of the cervix and vulva [35], and PDL1 overexpression confers resistance to radiation in HNSCC [36]. Notably, targeting PDL1 with antiPDL1 monoclonal antibody decreased PDL1 expression in a variety of tumors, including HNSCC [32,36,37]. Our results indicate that PDL1 and PDL2 are significantly overexpressed in samples with aUPD compared with those without aUPD. These findings provide potential insight into the mechanisms of PDL1 and PDL2 overexpression in HNSCC and support previous reports. Our results also suggest that identification of aUPD in defined regions may help to select patients for individualized therapy. TTF1, which encodes transcription termination factor 1 was also overexpressed in samples with aUPD in defined regions. TTF1 expression and blood vessel invasion were shown to correlate with PDL1 expression in sarcomatoid lung carcinoma [38]. Moreover, overexpression of TTF1 has been shown to be associated with poor prognosis in colorectal cancers [39].

Of note, we found that aUPD at the CDKN2A region was associated with OS in all samples and in HPVnegative samples, but not in HPVpositive samples. Indeed, expression of CDKN2A in samples with aUPD was significantly lower than in samples without aUPD. In contrast, deletion at the same region was not associated with OS or RFS in any of the sample groups. Other genes with significantly higher expression in samples with aUPD in defined regions compared with those without aUPD included mitochondrial genes (HINT2, ACAT1), genes involved in galactose (GALT, galactose1phosphate uridyltransferase) and carbohydrate metabolism (GBA2, glucosidase, beta [bile acid 2]), genes involved in nucleotide exchange (KIAA1432 and 9orf100), genes involved in oxidative stress defense (ERMP1, endoplasmic reticulum metalloprotease 1), ion channel and DNA excision repair genes (XPA, xeroderma pigmentosum, complementation group A), and nonreceptor tyrosine kinase gene (JAK2). In other studies, downregulation of ERMP1 and 9orf100 significantly reduced cell proliferation and migration [40–42]. CAT1 encodes a mitochondrially localized enzyme that catalyzes the reversible formation of acetoacetylCoA from two molecules of acetylCoA and regulates pyruvate dehydrogenase complex [43]. Inhibition of ACAT1 decreases cell proliferation and tumor growth [43].

In addition, VCP (valosincontaining protein), a member of the AAAATPase protein family, encodes a protein that interacts with other proteins to regulate endoplasmic reticulum–associated protein degradation. This involves multiple cellular functions during mitosis, including regulation of the spindle pole body, vesicular trafficking, and membrane fusion [44]. Expression of VCP, DCTN3, and STOML2 independently plays a role in increasing cell growth and anchorageindependent growth during the development of invasive oral carcinoma [45]. VCP also promotes growth, invasion, and metastasis in colorectal cancer through activation of STAT3 signaling [46]. Inhibition of VCP expression suppresses West Nile virus infection [47], as well as suppressing the cell cycle, inducing endoplasmic reticulum stress, and inducing caspasedependent cell death in ovarian cancer cells [48]. ANP32B encodes...
histone chaperone acidic nuclear phosphoprotein 32B (ANP32B) and plays an antiapoptotic role. Overexpression of ANP32B has been shown to lead to accumulation of henipavirus matrix (Hendra virus; HeV M) and nuclear proteins (Nipah virus; NiV M) [49]. Downregulation of ANP32B expression may enhance apoptosis in HNSCC cells. Our findings indicate that genes involved in galactose, carbohydrate and mitochondrial metabolism, nucleotide exchange, oxidative stress defense, and ion channels may also be involved in the pathogenesis of HNSCC, and inhibition of expression of these genes may reduce cell growth or invasion.

In summary, we demonstrated associations with patient outcome and identified genes in specific SOGs of a UPD that are differentially expressed in patients with a UPD, compared with patients without a UPD. These differentially expressed genes may be an indicator of tumor aggressiveness, and in turn affect survival. Therefore, a UPD analysis may be a useful tool to select targeted therapy for this heterogeneous disease.

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Conflicts of Interest

The authors declare no potential conflicts of interest.

Authors’ contributions

MT conceived and coordinated the study. MT and GBM designed the study. MT developed the methodology. MT, WL, CIA, GBM analyzed and interpreted the data; MT drafted the manuscript; MT, WL, CIA and GBM critically discussed the data and revised the manuscript. All authors read and approved final manuscript.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5–29.
2. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang MT, Goodman MT, Sibug-Saber M, Cozen W, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011;29:3294–301.
3. Patel SC, Carpenter WR, Tyres S, Couch ME, Weisler M, Hackman T, Hayes DN, Shores C, Chera BS. Increasing incidence of oral tongue squamous cell carcinoma in young white women, age 18 to 44 years. J Clin Oncol 2011;29:1488–94.
4. Leemans CR, Braakhuis BJ, Braekenhoff RH. The molecular biology of head and neck cancer. Nature Rev Cancer 2011;11:9–22.
5. Fitzgibbon J, Smith LL, Raghavan M, Smith ML, Debernardi S, Skoulakis S, Lillington D, Lister TA, Young BD. Association between acquired uniparental disomy and homoygous gene mutation in acute myeloid leukemias. Cancer Res 2005;65:9152–4.
6. Flotho C, Steinemann D, Mullighan CG, Neale G, Mayer K, Krazt CP, Schlegelberger B, Downing JR, Niemeyer CM. Genome-wide single-nucleotide polymorphism analysis in juvenile myelomonocytic leukemia identifies uniparental disomy surrounding the NFI locus in cases associated with neurofibromatosis but not in cases with mutant RAS or PTPN11. Oncogene 2007;26:5816–21.
7. Grand FH, Hidalgo-Curis CE, Ernst T, Zoi K, Zoi C, McGuire C, Kreil S, Jones A, Score J, Metzgeroth G, et al. Frequent CBL mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. Blood 2009;113:6182–92.
8. Martin-Trujillo A, Vidal E, Monteagudo-Sa N, Sanchez-Delgado M, Moran S, Hernandez Mora JR, Heyn H, Guitart M, Esteller M, Monk D. Copy number rather than epigenetic alterations are the major dictator of imprinted methylation in tumors. Nature Commun 2017;8:467.
9. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, Gittman K, Mathew S, Ma J, Pounds SB, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukemia. Nature 2007;446:578–64.
10. Tuna M, Ju Z, Smid M, Amos CI, Mills GB. Prognostic relevance of acquired uniparental disomy in serous ovarian cancer. Mod Cancer 2015;14:29.
11. Engel E. A new genetic concept: uniparental disomy and its potential effect, isodisomy. Am J Med Genet 1986;11:37–43.
12. Blackburn AC, McLary SC, Naeem R, Luszcz J, Stockton DW, Donehower M, Mohammed M, Mailhes JB, Soferr T, Naber SP, et al. Loss of heterozygosity occurs via mitotic recombination in Trp53+/ mice and associates with mammary tumor susceptibility of the BALB/c strain. Cancer Res 2004;64:5140–7.
13. Raghavan M, Lillington DM, Skoulakis S, Debernardi S, Chaplin T, Foot NJ, Lister TA, Young BD. Genome-wide single nucleotide polymorphism analysis reveals frequent partial uniparental disomy due to somatic recombination in acute myeloid leukemias. Cancer Res 2005;65:375–8.
14. Reliene R, Bishop AJ, Schlegelberger B, Brakenhoff RH. Involvement of homologous recombination in carcinogenesis. Adv Genet 2007;58:67–87.
15. Stephens K, Weaver M, Leppig KA, Manuyama K, Emanuel PD, Le Beau KM, Shannon KM. Interstitial uniparental isodisomy at clustered breakpoint intervals is a frequent mechanism of NF1 inactivation in myeloid malignancies. Blood 2006;108:1684–9.
16. Akuwujie I, Jones TJ, Munnane JP. Differences in the recruitment of DNA repair proteins at subtelomeric and interstitial I-SceI endonuclease-induced DNA double-strand breaks. DNA Repair 2017;49:1–8.
17. Cooi SW, Savera AT, Zarbo RJ, Benninger MS, Chase GA, Rybicki BA, Van Dyke DL. Prognostic implications of loss of heterozygosity at 8p21 and 9p21 in head and neck squamous cell carcinoma. Int J Cancer 2004;111:206–12.
18. Bockmuhl U, Schluns K, Kuchler I, Petersen S, Petersen I. Genetic imbalances with impact on survival in head and neck cancer patients. Am J Pathol 2005;167:369–75.
19. Graveland AP, Golusinski PJ, Buijze M, Douma R, Sons N, Kuik DJ, Bloemena E, Leemans CR, Braekenhoff RH, Braakhuis BJ. Loss of heterozygosity at 9p and p53 immunopositivity in surgical margins predict local relapse in head and neck squamous cell carcinoma. Int J Cancer 2011;128:1852–9.
20. Marasca GC, Capizzi C, Condorelli DF, Barlesi V. Genome-wide analysis of recurrent copy-number alterations and copy-neutral loss of heterozygosity in head and neck squamous cell carcinoma. J Oral Pathol Med 2014;43:20–7.
21. Morita T, Uzawa N, Mogushi K, Sumino M, Michikawa C, Takahashi KI, Myo T, Izumo T, Harada K. Characterizing genetic transitions of copy number alterations and allelic imbalances in oral tongue carcinoma metastasis. Genes Chromosomes Cancer 2016;55:975–86.
22. Purdie KJ, Lambert SR, Teh MT, Chaplin T, Molloy G, Raghavan M, Kelhell LM, Leigh IM, Harwood CA, Proby CM, et al. Allelic imbalances and microdeletions affecting the PTPRD gene in cutaneous squamous cell carcinomas detected using single nucleotide polymorphism microarray analysis. Genes Chromosomes Cancer 2007;46:661–9.
23. Gondek LP, Tiu R, O’Keefe CL, Sekeres MA, Theil KS, Maciejewski JP. Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS. MDSMPID, and MDS-derived AML. Blood 2008;111:1534–42.
24. Tuna M, Amos CI, Mills GB. Genome-wide analysis of head and neck squamous cell carcinomas reveals HPV, TP53, smoking and alcohol-related allele-based acquired uniparental disomy genomic alterations. Neoplasia 2019;21:197–205.
25. Network T. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015;517:576–82.

26. Nulton TJ, Olex AL, Dozmorov M, Morgan IM, Windle B. Analysis of The Cancer Genome Atlas sequencing data reveals novel properties of the human papillomavirus 16 genome in head and neck squamous cell carcinoma. Oncotarget 2017;8:17684–99.

27. Yamamoto G, Nannya Y, Kato M, Sanada M, Levine RL, Kawamata N, Hangaiishi A, Kurokawa M, Chiba S, Gilliland DG, et al. Highly sensitive method for genomewide detection of allelic composition in nonpaired, primary tumor specimens by use of affymetrix single-nucleotide-polymorphism genotyping microarrays. Am J Human Genet 2007;81:114–26.

28. L.M. McShane, D.G. Altman, W. Sauerbrei, S.E. Taube, M. Gion, G.M. Clark, N.C.I.E.W.G.o.C.D. Statistics Subcommittee of, REporting recommendations for tumor MARKer prognostic studies (REMARK), Breast Cancer Res Treat, 100 (2006) 229–235.

29. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B Met 1995;57:289–300.

30. Franzen A, Vogt TJ, Muller T, Dietrich J, Schrock A, Golletz C, Brossart P, Booz F, Landsberg J, Kristiansen G, et al. PD-1-L1 (CD274) and PD-L2 (PDCD1LG2) promoter methylation is associated with HPV infection and transcriptional repression in head and neck squamous cell carcinomas. Oncotarget 2018;9:641–50.

31. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nature Rev Cancer 2016;16:275–87.

32. Yu GT, Bu LL, Huang CF, Zhang WF, Chen WJ, Gurkind JS, Kulkarni AB, Sun ZJ. PD-1 blockade attenuates immunosuppressive myeloid cells due to inhibition of CD47/SIRPα axis in HPV negative head and neck squamous cell carcinoma. Oncotarget 2015;6:42067–80.

33. The Cancer Genome Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:202–9.

34. The Cancer Genome Network. Integrated genomic and molecular characterization of cervical cancer. Nature 2017;543:378–84.

35. Howitt BE, Sun HH, Roemer MG, Kelley A, Chapuy B, Aviki E, Pak C, Connelly C, Giini E, Shi Y, et al. Genetic basis for PD-L1 expression in squamous cell carcinomas of the cervix and vulva. JAMA 2016;2:518–22.

36. Skinner HD, Giri U, Yang LP, Kumar M, Liu Y, Story MD, Pickering CR, Byers LA, Williams MD, Wang J, et al. Integrative analysis identifies a novel AXL-PI3 kinase-PD-L1 signaling axis associated with radiation resistance in head and neck cancer. Clin Cancer Res 2017;23:2713–22.

37. Kotakis A, Georgoulas V, Avedumab, an anti-PD-L1 monoclonal antibody, shows activity in various tumour types. Lancet Oncol 2017;18:556–7.

38. Vicira T, Antoine M, Hamard C, Fallet V, Durnisssaux M, Rabbe N, Rodenas J, Cadranel J, Widelx M. Sarcomatoid lung carcinomas show high levels of programmed death ligand-1 (PD-L1) and strong immune-cell infiltration by TCD3 cells and macrophages. Lung Cancer 2016;98:51–8.

39. Ueda M, Iguchi T, Nambara S, Saito T, Komatsu H, Sakimura S, Hirata H, Uchi R, Takano Y, Shiden Y, et al. Expression of transcription termination factor 1 is associated with a poor prognosis in patients with colorectal cancer. Ann Surg Oncol 2015;22(Suppl 3):S1490–8.

40. Siniossoglou S, Peak-Chew SY, Pelham HR. Ric1p and Rgp1p form a complex that catalyzes nucleotide exchange on Ypt6p. EMBO J 2000;19:4885–94.

41. Bensen ES, Yeung BG, Payne GS. Ric1p and the Ypt6p GTPase function in a common pathway required for localization of trans-Golgi network membrane proteins. Mol Biol Cell 2001;12:13–26.

42. Grandi A, Santì A, Campagnoli S, Pari M, De Camilli E, Song C, Jin B, Lacombe A, Castori-Eppenberger S, Sarmentos P, et al. ERMP1, a novel potential oncogene involved in UPR and oxidative stress defense, is highly expressed in human cancer. Oncotarget 2016;7:63596–610.

43. Fan J, Lin R, Xia S, Chen D, Elf SE, Liu S, Pan Y, Xu H, Qian Z, Wang M, et al. Tetrameric acetyl-CoA acetyltransferase 1 is important for tumor growth. Mol Cell 2016;64:859–74.

44. Yang FC, Lin YH, Chen WH, Huang JY, Chu HY, Su WH, Wang HT, Chiang CY, Hsu PH, Tsai MD, et al. Interaction between salt-inducible kinase 2 (SIK2) and p97/valosin-containing protein (VCP) regulates endoplasmic reticulum (ER)-associated protein degradation in mammalian cells. J Bio Chem 2015;288:33861–72.

45. Towle R, Tsui IF, Zhu Y, MacLellan S, Poh CF, Garnis C. Recurring DNA copy number gain at chromosome 9p13 plays a role in the activation of multiple candidate oncogenes in progressing oral premalignant lesions. Cancer Med 2014;3:1170–84.

46. Fu Q, Jiang Y, Zhang D, Liu X, Guo J, Zhao J. Valosin-containing protein (VCP/p97) plays a role in the replication of West Nile virus. Virus Res 2017;228:114–23.

47. Bastola P, Neums L, Schoenen FJ, Chien J. VCP inhibitors induce endoplasmic reticulum stress, cause cell cycle arrest, trigger caspase-mediated cell death and synergistically kill ovarian cancer cells in combination with Salubrinal. Mol Oncol 2016;10:1559–74.

48. Bauer A, Neumann S, Karger A, Henning AK, Maisner A, Lamp B, Dietzel E, Kwasnitschka L, Balkema-Buschmann A, Kell GM, et al. ANP32B is a nuclear target of henipavirus M proteins. PloS one 2014;9:e97233.

49. Shen SM, Yu Y, Wu YL, Cheng JK, Wang LS, Chen GQ. Downregulation of ANP32B, a novel substrate of caspase-3, enhances caspase-3 activation and apoptosis induction in myeloid leukemic cells. Carcinogenesis 2010;31:419–26.