IN SILICO GENE EXPRESSION ANALYSIS OF CRUCIAL CELL CYCLE CONTROL GENE CDKN2A AND CDKN2B IN HEAD AND NECK SQUAMOUS CELL CARCINOMA.

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy with poor prognosis. Dysfunction of the regular role of the cell cycle results in the progression of cancer. Cellular programs like proliferation, differentiation, senescence, and apoptosis are affected in cancer. The present study was aimed at determining the expression of CDKN2A and CDKN2B proteins which are principal regulators of the cell cycle. This study was conducted using the clinical data of HNSCC collected from The Cancer Genome Atlas database (TCGA). This study included 564 tissue samples, of which 520 were of patients with primary HNSCC tumors and 44 were normal. In the present study, we used the UALCAN database to analyse CDKN2A and CDKN2B expressions. The results showed that CDKN2A (p<1e-12) and CDKN2B (p=5.025e-04) were highly expressed in HNSCC as compared to normal tissues. Analysis of effects of CDKN2A expression levels on HNSCC patients shows high expression in 130 cases and low / medium expression in 389 cases. Analysis of survival curve based on high level and low level expression of CDKN2B revealed that high level expression of CDKN2A conferred survival advantages to patients with HNSCC than those presenting with a low level expression pattern. In conclusion, CDKN2A and CDKN2B were highly expressed in HNSCC and CDKN2B associated with good prognosis in HNSCC patients. Therefore, CDKN2A and CDKN2B may serve as
therapeutic targets for HNSCC.

KEYWORDS: CDKN2A, CDKN2B, HNSC, survival, cell cycle


INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of oral cavity which is frequently associated with poor prognosis (Ren et al., 2015; Sharma 2018; Jangid et al., 2015). Prevalence of oral cancer is reported as 45% in India and 10% in Pakistan (Pentenero, 2005). The parameters such as age, genders, habits, race, socioeconomic status, clinical parameters (like Staging of lesion, location of lesion) and histological parameters (perivascular, perineural, grading, pattern of invasion, tumour thickness) play important role in diagnosis, prognosis and further treatment planning (Pentenero, 2005). It is known that the tumor cell during development undergoes molecular alterations in several cellular molecules including DNA, RNA, and proteins which could be attributed to the inherent biological properties of the cancer cell (Sridharan, 2017; Gheena, 2019). The upregulation of genes is noted in extracellular matrix degradation and epithelial to mesenchymal transition, the down regulation of genes are found in detoxification pathways (Kumar et al., 2015; Thangaraj et al., 2016), also showed stromal alterations (Jayaraj et al., 2015). Molecular studies show involvement of preneoplastic cells (Gupta and Ramani, 2016). Recent studies have shown viruses like human papilloma viruses involved in oral cancers (Gifrina et al., 2015; Vivekaet al., 2016). Premalignant conditions like leukoplakia, oral epithelial dysplasia, and oral submucous fibrosis also reported with malignant transformation (Gifrina et al., 2015; Jayaraj et al., 2015; Viveka et al., 2016; Sridharan, 2017). Patients with OSCC are diagnosed at the later stages leading to decreases in survival rate (Hema et al., 2019). Latest report from International agency for research on cancer (IARC); Lip Oral cavity in 2018 reports show incidence of 3,54,864 around the world in which 119,992 cases were reported in India. The prevalence report shows 9,13,514 cases around the world in which 9,72,616 cases were reported in India.

Cellular programs like proliferation, differentiation, senescence, and apoptosis are affected in cancer (Todd et al., 2002; Deepak et al., 2019). CDKN2A is a gene located in the human chromosome at band p21.3, which shows ubiquitously expressed proteins like P16 and P14arf which act as tumor suppressors by regulating the cell cycle. P16 inhibits Cyclin dependent kinases 4 and 6 which activates the retinoblastoma finally blocks G1 to S phasetransition, P14arf protein activates the p53 tumor suppressor (Smedset et al., 2002). CDKN2B is a gene located in chromosome 9p21 forms a complex with CDK4/CDK6 which prevents the activation of CDK kinases that helps incontrol of G1 progression in a progress cycle (Iolascon et al., 1998, Sivaramakrishnan and Ramani, 2015; Hannah et al., 2018). Previous studies have shown somatic alterations in the CDKN2AandCDKN2B geneoccurring in many cancer type and germ linemutation carriers (Deepak et al., 2019). Swati et al in 2017 reported that there is direct involvement of CDKN2A/P16 in oral cancer and down regulation in recurrent cases (Padhi et al., 2017; Deepak et
There are not many studies pertaining to the cell cycle control proteins which are principal regulators of cell cycle. Hence in present study shows gene expression analysis of cancer cell cycle control genes CDKN2A and CDKN2B in HNSCC.

MATERIALS AND METHODS

Gene expression and survival analysis using UALCAN database

This study was conducted using the clinical and gene expression data of HNSCC from TCGA database. This study includes 564 tissue samples, of which 520 were primary HNSCC tumors and 44 were normal tissue samples. In the present study, the UALCAN database (http://ualcan.path.uab.edu/) was used to analyse CDKN2A and CDKN2B expressions in primary HNSCC and normal tissues. We also used the UALCAN to find patient survival information in HNSCC based on CDKN2A and CDKN2B genes expression. Transcripts per million (TPM) is a normalization method for RNA-seq data. The TPM values used for the generation of box-whisker plots were also used to determine the significant difference between the groups. The t test was performed using PERL script with the Comprehensive Perl Archive Network (CPAN) module. Combined survival effect analysis of gene expression and other clinical parameters such as race, gender, tumor grade, cancer subtypes were assessed using multivariate Kaplan-Meier survival analysis.

RESULTS AND DISCUSSION

The CDKN2A and CDKN2B expression analysis using TCGA dataset revealed a significant difference between HNSCC and normal tissues. Both CDKN2A (p<1e-12) and CDKN2B (p=5.025e-04) were highly expressed in HNSCC and compared with normal tissues (figure 1 and figure 2). In this study, the expression of CDKN2A was significantly higher in male rather than females (p=0.001) (Figure 3). In addition, our result showed that CDKN2A was highly expressed in HPV positive patients compared with HPV negative patients (p=1.110e-12) (Figure 4). Moreover, analysis of survival curve based on high level and low level expression of CDKN2A revealed that high level expression of CDKN2A conferred survival advantages to patients with HNSCC than those having a low level expression pattern (p=0.0003) (Figure 5). Previous study shows somatic alterations in the CDKN2A and CDKN2B gene occur in many cancer types (Swathy, 2015; Deepak et al., 2019). In another study detection of genetic and epigenetic alterations in CDKN2A and P53 promoted by hypermethylation of CDKN2A which alteration detected.

Examination of intragenic mutation of CDKN2A and CDKN2B studied on 60 esophageal carcinoma resulted in single CDKN2B nonsense mutation occurring among 21 adenocarcinomas (5%), substitution of T for G was seen at nucleotide 440 (codon 35), resulting in the replacement of glutamic acid by a stop codon, G to T transversion was seen only in somatic tumor DNA. One (3%) of the 39 squamous tumors showed CDKN2B mutation, which does not change the encoded amino acid, this alteration was a substitution of T for C at position 697 (codon 120), again occurring in tumor and absent in homologous normal tissue (Suzuki et al., 1995). Previous study reported point mutations of CDKN2A, observed in human melanoma cell lines, esophageal and pancreatic carcinomas and few.
mutations in CDKN2B coding region (Cairns et al., 1994; Suzuki et al., 1995). Other study with esophageal squamous cell carcinoma, allelic losses were determined at the CDKN2A locus at 9p21 and at the p53 locus at 17p13.1 by analysing polymorphic dinucleotide microsatellite markers in tumours and corresponding benign tissues (Smeds et al., 2002). In HNSCC, CDKN2A the second most frequently mutated gene after TP53, shows absence of protein overexpression, homozygous deletion, and promoter methylation (Stransky et al., 2011; Lim et al., 2014). In conclusion, CDKN2A and CDKN2B were highly expressed in HNSCC. In addition, high expression of CDKN2B was associated with good prognosis in HNSCC patients. Therefore, CDKN2A and CDKN2B may serve as therapeutic targets for HNSCC.

CONCLUSION:

Cell cycle regulators serve as checkpoints in deciding the fate of a cell. Extensive lesions in the DNA are sensed by tumor suppressor genes which tend to arrest cell cycle from proceeding. Any dysregulation of these checkpoint molecules invariably lead to uncontrolled proliferation of cells, which is associated with the development and progression of tumors. The present study elucidates the possible association of two crucial genes viz., CDKN2A and CDKN2B related to cell cycle process, with HNSCC. The results obtained through computational analysis provide insights into the key role played by these genes in the development of disease phenotype. Hence, further experimental validation of these genes has to be carried out to provide concrete evidence on their association with HNSC.

AUTHOR CONTRIBUTION:

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Figure 1. Box-whisker plot showing relative expression of CDKN2A in normal and primary tumor of HNSC patients. The x axis - denotes sample types and y axis - denotes mRNA counts expressed as TPM (transcripts per million). The expression of CDKN2A (p < 10^{-12}) showed a significant difference in expression between the normal (blue) and HNSC (red) sample types. A p value less than 0.05 is considered to be significant.
Figure 2. Box-whisker plot showing relative expression of $CDKN2B$ in normal and primary tumor of HNSC patients. The x axis - denotes sample types and y axis - denotes mRNA counts expressed as TPM (transcripts per million). The expression of $CDKN2B$ ($p = 5.02 \times 10^{-4}$) showed a significant difference in expression between the normal (blue) and HNSC (red) sample types. A p value less than 0.05 is considered to be significant.
Figure 3. Box-whisker plot showing relative expression of *CDKN2A* male and female HNSC patients in comparison to normal tissue samples. The x axis - denotes gender and y axis - denotes mRNA counts expressed as TPM (transcripts per million). The expression of *CDKN2A* (p = 0.001) showed a significant difference in expression between male (red) and female (orange) patients relative to normal tissues (blue). A p value less than 0.05 is considered to be significant.
Figure 4. Box-whisker plot showing relative expression of CDKN2A in normal and subgroups of HNSC patients stratified based on HPV status. The x axis - denotes HPV status in patients and normal tissues. The y axis - denotes mRNA counts expressed as TPM (transcripts per million). The expression of CDKN2A ($p = 1.1 \times 10^{-12}$) showed a significant difference in gene expression between HPV positive (red) and HPV negative (orange) patients relative to normal samples (blue). A $p$ value less than 0.05 is considered to be significant.
Figure 5. Kaplan–Meier plots showing the association of *CDKN2A* expression on patients’ survival. The x-axis represents time in days and y-axis shows the probability of surviving. The patients with high levels of expression (red) were found to have a survival advantage over the patient’s presenting with low levels of expression (blue) of *CDKN2A* (*p* = 0.00038).