Deciphering the Genetic Aberrations in DNA Damage Response Genes and Their Possible Association with HNSCC

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Authors’ contributions

This work was carried out in collaboration among all authors. Author JVP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ASSG and AP managed the analyses of the study. Author LA managed the literature searches and performed certain computational analysis. All authors read and approved the final manuscript.

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ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) includes carcinomas in the oral cavity, pharynx and larynx. It is considered as the sixth most common form of cancer in the world. Several studies have confirmed that smoking and alcohol consumption are the major risk factors for HNSCC. DNA damage response genes play an important role in the maintenance of the genome. Defects in cell

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cycle checkpoint and DNA repair mechanisms, such as mutation or abnormalities, may lead to the wide spectrum of human diseases. The present study employs databases and computational tools to identify the genetic abnormalities associated with DNA damage related genes which might have a direct or indirect association with HNSCC. The demographic details of HNSCC patients was obtained from The Cancer Gene Atlas (TCGA, Firehose Legacy) dataset hosted by the cBioportal database. The oncoprint data analysis revealed the highest frequency of gene alteration in the ATR gene (15%), followed by ATM, BRCA2 and CHEK2 (5%). Other genes showed less than 5% alteration. The gene expression profile of ATR gene revealed its differential expression pattern in different grades of tumor relative to normal samples. The survival curve analysis using Kaplan-Meier method revealed that a high level expression of the ATR gene leads to poor survival rate in the female HNSCC patients when compared to males. Thus the present study has identified gross and single nucleotide variants in the ATR gene which could have a putative role in the development of tumor. Further experimental research is required to confirm this association.

Keywords: Carcinoma; DNA damage response genes; mutations; cell cycle checkpoints; HNSCC.

1. INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) includes carcinomas in the oral cavity, pharynx and larynx. It is considered as the sixth most cancer in the world. It’s annual mortality rate was about 50%. HNSCC can be widely classified based on its location, including oral cavity, oropharynx, nasal cavity and air sinuses [1]. The factors associated with the development of HNSCC were mostly related to environmental components and viral agents. Several studies have confirmed that smoking and alcohol consumption are the major risk factors associated with HNSCC [2]. Another implicated risk factor is human papilloma virus (HPV) infection which particularly affects the oropharynx [3]. Epidemiological studies suggest that there is an increase in the incidence of oropharyngeal cancers caused by human papillomavirus [4]. DNA damage response genes play an important role in the maintenance of a healthy genome. Defects in cell cycle checkpoint or in DNA repair mechanisms, such as mutation or abnormalities, may lead to the wide spectrum of human diseases [5]. Dysregulation or mutation of DNA repair genes can affect the genomic stability, induce aging and other forms of immune deficiencies and cancer [6]. Several studies have reported that upregulation of DNA repair genes confer resistance to chemotherapy and radiotherapy [7]. The study has been reported that inhibitors of DNA pathways have the high potential to sensitize tumour inducing cells in the patient’s affected with cancers [8]. Other than DNA repair pathways, these genes are also involved in nucleotide excision repair, mismatch repair, non-homologous and homologous recombination, which are found liable to all types of cancers [9]. DNA repair defects also cause various inherited defects such as aging syndromes including Ataxia telangiectasia, Nijmegen syndrome, Werner syndrome, Bloom syndrome, Xeroderma pigmentosum [10]. A study evidenced that DNA repair genes show metastasis with the onset of tumorigenesis [11]. In view of the above facts, the study has been designed to identify genetic variations which could potentiate the development of tumors.

2. MATERIALS AND METHODS

2.1 Sample Data Set

The present study follows a retrospective observational study design. The source of the patient's data was collected from the cBioportal database. This database provides an exhaustive collection of patient's details from different cohorts. The TCGA, Firehose legacy data set consisted of 528 head and neck squamous cell carcinoma cases with sequencing and number of alteration data available for 504 tumor samples [12,13]. A complete profile of mutated, amplified, deleted genes was available for each case in the dataset. The demographic details of the cases in the dataset have been provided in (Table 1). The list of twelve DNA damage response genes are available in the cBioportal database which included CHEK 1, CHEK 2, RAD 51, BRCA1, BRCA2, MLH 1, MLH2, ATM, ATR, MDC1, PARP1, FANC, FANC. The user defined queries based on these genes returned an oncoprint data which was used for further analysis.

2.2 Oncoprint Data Analysis

The Oncoprint data analysis provided information on the type of gene alterations viz., gene amplification, deep deletion, mutations/variants.

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The percentage alteration and frequency of variant allele were provided for the several DNA damage response genes. These details were used to derive a putative association between the disease phenotype and genotype, to identify the variations in less understood DNA pathways or genes which could relate to HNSCC development and progression (Table 2).

2.3 Pathogenicity Analysis

PROVEAN (Protein Variation Effect Analyzer) is a computational tool used to assess the pathogenicity of a single nucleotide variant, especially the mis-sense type. The reference protein sequences of all the genes encoding proteins selected in the study were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/protein). Based on the scores obtained the pathogenicity of the variant was determined (Table 2). A score less than -2.5 or greater than -2.5 considered to be as deleterious and neutral respectively [14].

2.4 Gene Expression and Survival Curve Analysis

The expression of the gene in HNSCC was analysed using the UALCAN (http://ualcan.path.uab.edu/cgi-bin/TCGA-survival1.pl?genenam=) database. Survival curve analysis based on the gender and expression profile was performed to demonstrate the putative role of ATR gene with HNSCC. Gene expression data is expressed as transcripts per million (TPM). Kaplan-Meier survival analysis was performed to identify the prognostic value of the gene with highest frequency of genetic alteration [15].

3. RESULTS AND DISCUSSION

Genomic instability is the significant feature of cancer cells. It is caused by down regulation of the DNA damage response pathways which is controlled by ATM, Rad3 and ATR genes [16]. Many studies have performed systematic analysis on various types of DNA damage response genes. The RAD9 gene functions as part of a heterotrimer with RAD1 gene, which is involved in apoptosis and controller of cell cycle checkpoints in DNA damage pathways [17,18]. The present study was intended to identify genetic alterations in DNA damage genes in HNSCC patients. The frequency of genetic alterations ranged from 0.4 to 15%. Among the various DNA damage response genes, the ATR gene showed the highest frequency (15%) of gene alteration. The frequency of genetic alteration in other genes were as follows: CHEK1 (1.2%), CHEK2 (5%), RAD51 (0.4%), BRCA1 (2.6%), BRCA2 (5%), MLH1 (1.8%), MLH2 (1.6%), ATM (5%), PARP1 (2%), FANCF (1%) and MDC1 (4%) (Fig. 1; Table 2). Gene amplification and mutations

Table 1. Demographic details of patients analysed in the present study (as obtained from the cBioportal site)

| Gender         | Male (n = 386) |
|----------------|---------------|
|                | Female (n = 142) |
| Mutation count | 6-3181        |
| Diagnosis age  | 19-90 years   |
| Smoking status | Smokers: 515   |
|                | Data not available: 12 |
|                | Unknown: 1     |
| Alcohol history| Yes – 352      |
|                | No – 165       |
| Neoplasm Histologic grade | Grade 1: 63 |
|                | Grade 2: 311   |
|                | Grade 3: 125   |
|                | Grade 4: 7     |
|                | Grade GX: 18   |
| Race category  | White: 452     |
|                | African: 48    |
|                | Asian: 11      |
|                | American Indian or Alaska native: 2 |
|                | Data not available: 15 |
Table 2. The frequency of genetic alterations, cytogenetic location of the gene, protein encoded by genes, variant allele frequency in tumor sample for the DNA damage response family of genes

| Gene     | Protein                          | Alteration                        | Cytogenetic location | % of alteration | Variant allele frequency in tumor sample |
|----------|----------------------------------|-----------------------------------|----------------------|-----------------|------------------------------------------|
| CHEK1    | Checkpoint Kinase 1              | Gene Amplification                | 11q24.2              | 1.2             |                                          |
|          |                                  | Deep Deletion                     |                      |                 | 0.1                                      |
|          |                                  | R379*                             |                      |                 |                                          |
| CHEK2    | Checkpoint Kinase 2              | Gene Amplification                | 22q12.1              | 1.8             |                                          |
|          |                                  | T323Lfs*14                        |                      |                 | 0.45                                     |
|          |                                  | N166S                             |                      |                 | 0.07                                     |
|          |                                  | T532I                             |                      |                 | 0.21                                     |
|          |                                  | S372F                             |                      |                 | 0.26                                     |
| RAD51    | RAD51 Recombinase                | Gene Amplification                | 15q15.1              | 0.6             |                                          |
|          |                                  | Deep Deletion                     |                      |                 | 0.45                                     |
|          |                                  | S296L                             |                      |                 |                                          |
| BRCA1    | BRCA1 DNA Repair Associated      | Gene Amplification                | 17q21.31             | 2.6             |                                          |
|          |                                  | X101_Splice                       |                      |                 | 0.37                                     |
|          |                                  | D853N                             |                      |                 | 0.27                                     |
|          |                                  | H816Y                             |                      |                 | 0.05                                     |
|          |                                  | G1492R                            |                      |                 | 0.23                                     |
|          |                                  | E230Q                             |                      |                 | 0.38                                     |
|          |                                  | D1505N                            |                      |                 | 0.22                                     |
|          |                                  | R664G                             |                      |                 | 0.14                                     |
|          |                                  | R1645M                            |                      |                 | 0.47                                     |
|          |                                  | R1737T                            |                      |                 | 0.12                                     |
|          |                                  | S184C                             |                      |                 | 0.04                                     |
|          |                                  | K175N                             |                      |                 | 0.14                                     |
| BRCA2    | BRCA 2 DNA Repair Associated     | Deep deletion                     | 13q13.1              | 4               |                                          |
|          |                                  | Y2884*                            |                      |                 | 0.20                                     |
|          |                                  | R2625*                            |                      |                 | 0.26                                     |
|          |                                  | Q2749*                            |                      |                 | 0.35                                     |
|          |                                  | Y839Ifs*42                        |                      |                 | 0.10                                     |
|          |                                  | E2175Q                            |                      |                 | 0.25                                     |
| Gene        | Protein                           | Alteration                          | Cytogenetic location | % of alteration | Variant allele frequency in tumor sample |
|-------------|-----------------------------------|--------------------------------------|----------------------|-----------------|------------------------------------------|
|             |                                   |                                      |                      |                 |                                          |
|            | E3342K                            |                                      |                      | 0.27            |                                          |
|            | G500V                             |                                      |                      | 0.43            |                                          |
|            | V1605D                            |                                      |                      | 0.05            |                                          |
|            | E1571K                            |                                      |                      | 0.60            |                                          |
|            | I2105V                            |                                      |                      | 0.44            |                                          |
|            | E2903K                            |                                      |                      | 0.16            |                                          |
|            | P606L                             |                                      |                      | 0.13            |                                          |
|            | K3315N                            |                                      |                      | 0.38            |                                          |
|            | S3218F                            |                                      |                      | 0.07            |                                          |
|            | R2861T                            |                                      |                      | 0.25            |                                          |
|            | F312C                             |                                      |                      | 0.33            |                                          |
|            | S3231L                            |                                      |                      | 0.23            |                                          |
|            | D1386N                            |                                      |                      | 0.27            |                                          |
|            | E3393Q                            |                                      |                      | 0.18            |                                          |
|            | S3123G                            |                                      |                      | 0.13            |                                          |
|            | P1039H                            |                                      |                      | 0.13            |                                          |
| MLH1       | MutL Homolog 1                    | Deep deletion                        | 3p22.2               | 1.4             |                                          |
|            |                                   |                                      |                      |                 |                                          |
|            | E102*                             |                                      |                      | 0.56            |                                          |
|            | 1262M                             |                                      |                      | 0.28            |                                          |
|            | A281V                             |                                      |                      | 0.07            |                                          |
|            | P138R                             |                                      |                      | 0.43            |                                          |
|            | V647L                             |                                      |                      | 0.13            |                                          |
| MLH2       | DNA Mismatch repair protein       | Deep deletion                        | 2q32.2               | 1.8             |                                          |
|            | Msh2                              |                                      |                      |                 |                                          |
|            | A230Lfs*16                        |                                      |                      | 0.08            |                                          |
|            | G162V                             |                                      |                      | 0.23            |                                          |
|            | R382C                             |                                      |                      | 0.19            |                                          |
|            | A107T                             |                                      |                      | 0.29            |                                          |
|            | S142L                             |                                      |                      | 0.13            |                                          |
|            | A434V                             |                                      |                      | 0.16            |                                          |
|            | Q314R                             |                                      |                      | 0.27            |                                          |
| ATM        | ATM Serine/Threonine Kinase       | Gene Amplification                   | 11q22.3              | 5               |                                          |
|            |                                   | Deep Deletion                        |                      |                 |                                          |
|            |                                   | R337C                                |                      |                 |                                          |

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| Gene          | Protein                  | Alteration | Cytogenetic location | % of alteration | Variant allele frequency in tumor sample |
|---------------|--------------------------|------------|----------------------|-----------------|------------------------------------------|
|               | X1726_splice             |            |                      |                 |                                          |
|               | X25_splice               |            |                      |                 |                                          |
|               | 12899M                   |            |                      | 0.20            |                                          |
|               | Y1248C                   |            |                      | 0.42            |                                          |
|               | D1053N                   |            |                      | 0.27            |                                          |
|               | 1238V                    |            |                      | 0.22            |                                          |
|               | I1035V                   |            |                      | 0.30            |                                          |
|               | W412C                    |            |                      | 0.28            |                                          |
|               | V1238              |            |                      | 0.16            |                                          |
|               | L1035V                   |            |                      | 0.54            |                                          |
|               | W412C                    |            |                      | 0.39            |                                          |
|               | K2749N                   |            |                      | 0.28            |                                          |
|               | D2997N                   |            |                      | 0.16            |                                          |
|               | E2932K                   |            |                      | 0.07            |                                          |
|               | D2988N                   |            |                      | 0.11            |                                          |
|               | V1506M                   |            |                      | 0.12            |                                          |
|               | E2238Q                   |            |                      | 0.33            |                                          |
|               | T2333I                   |            |                      | 0.24            |                                          |
|               | T2754A                   |            |                      | 0.11            |                                          |
|               | H636N                    |            |                      |                 |                                          |
| ATR           | ATR Serine/Threonine Kinase | Gene Amplification | 3q23              | 10              |                                          |
|               | Y2637                    |            |                      | 0.14            |                                          |
|               | W1800R                   |            |                      | 0.35            |                                          |
|               | S1348F                   |            |                      | 0.10            |                                          |
|               | M2266I                   |            |                      | 0.15            |                                          |
|               | S61C                      |            |                      | 0.10            |                                          |
|               | H1684D                   |            |                      | 0.07            |                                          |
|               | E2373V                   |            |                      | 0.19            |                                          |
|               | E560K                    |            |                      | 0.12            |                                          |
|               | P2549T                   |            |                      | 0.51            |                                          |
|               | G2319E                   |            |                      | 0.25            |                                          |
|               | I693M                    |            |                      | 0.10            |                                          |
|               | E54Q                      |            |                      | 0.06            |                                          |
|               | N2199S                   |            |                      | 0.27            |                                          |
|               | V66M                      |            |                      | 0.16            |                                          |
|               | S549C                    |            |                      | 0.40            |                                          |
| Gene               | Protein                                      | Alteration          | Cytogenetic location | % of alteration | Variant allele frequency in tumor sample |
|--------------------|----------------------------------------------|---------------------|----------------------|-----------------|------------------------------------------|
|                    |                                               | F99L                |                      | 0.27            |                                          |
|                    |                                               | S246F               |                      | 0.10            |                                          |
|                    |                                               | L400F               |                      | 0.06            |                                          |
|                    |                                               | L987I               |                      | 0.17            |                                          |
|                    |                                               | R215S               |                      | 0.09            |                                          |
|                    |                                               | V1802A              |                      | 0.13            |                                          |
|                    |                                               | D1243H              |                      | 0.10            |                                          |
| MDC 1              | Mediator Of DNA Damage Checkpoint 1           | Gene Amplification  | 6p21.33              | 4               |                                          |
|                    |                                               | E459K               |                      | 0.21            |                                          |
|                    |                                               | S1253Y              |                      | 0.19            |                                          |
|                    |                                               | S1743C              |                      | 0.21            |                                          |
|                    |                                               | D1175Y              |                      | 0.29            |                                          |
|                    |                                               | L122F               |                      | 0.71            |                                          |
|                    |                                               | D1845N              |                      | 0.28            |                                          |
|                    |                                               | S1440F              |                      | 0.05            |                                          |
|                    |                                               | R114H               |                      | 0.28            |                                          |
|                    |                                               | D134V               |                      | 0.25            |                                          |
|                    |                                               | Q1056*              |                      | 0.14            |                                          |
|                    |                                               | H354Y               |                      | 0.14            |                                          |
|                    |                                               | N1397I              |                      | 0.28            |                                          |
|                    |                                               | G1401C              |                      | 0.08            |                                          |
|                    |                                               | H368N               |                      | 0.11            |                                          |
| PARP1              | Poly(ADP-Ribose) Polymerase 1                | Gene Amplification  | 1q42.12              | 1.4             |                                          |
|                    |                                               | S274F               |                      | 0.23            |                                          |
|                    |                                               | E456Q               |                      | 0.27            |                                          |
|                    |                                               | P174T               |                      | 0.54            |                                          |
| FANCF              | FA Complementation Group F                   | L86F                | 11p14.3              | 0.4             | 0.33                                      |
|                    |                                               | A250D               |                      |                 | 0.29                                      |
(truncating and non-synonymous) were found in the ATR gene. The PROVEAN predictions of genes reported both neutral and deleterious outcomes across the CHEK2, RAD51, BRCA1, BRCA2, ATR, ATM group of DNA damage response genes (Table 3).

The gene expression profile observed in HNSCC patients could be related to the gene amplification documented earlier (Fig. 2). The comparison of gene expression patterns between different grades of HNSC returned significant values between normal and different grades of tumor viz., grade 1, grade 2 grade 3. Significant difference in gene expression was observed between different grades also (Fig. 3). Furthermore, the survival probability analysis employing Kaplan–Meier plots showed significant association of ART gene expression in combination with the gender with HNSCC patient’s survival. The high level expression of the ATR gene in females in comparison to male patients provided a significant result with a p value = $<10^{-12}$. The differential gene expression pattern and the associated survival probability in male and female HNSCC patients is suggestive of the prognostic significance of the gene (Fig. 3).

![Fig. 1. On coprint data depicting gene alterations in the DNA damage response genes. Each of the grey bars represent HNSCC patients](image)

![Fig. 2. Box-Whisker plot showing relative expression profile of ART gene in primary tumor of HNSC patients in comparison to normal samples (p value = $<10^{-12}$). The X axis denotes the TCGA samples and Y axis denotes the transcripts per million values. A p value less than 0.05 is considered to be significant](image)
Table 3. Pathogenicity of missense variants identified in DNA damage response genes as predicted by PROVEAN

| Gene  | Alteration | PROVEAN Score | PROVEAN Prediction |
|-------|------------|---------------|--------------------|
| CHEK2 | N166S      | -4.818        | Deleterious        |
|       | T532I      | -0.767        | Neutral            |
|       | S372F      | -5.551        | Deleterious        |
| RAD51 | S296L      | -5.658        | Deleterious        |
| BRCA1 | D853N      | -4.562        | Deleterious        |
|       | H816Y      | -4.582        | Deleterious        |
|       | G1492R     | -1.206        | Neutral            |
|       | E230Q      | -0.553        | Deleterious        |
|       | D1505N     | -0.312        | Neutral            |
|       | R664G      | -5.388        | Deleterious        |
|       | R1645M     | -0.520        | Neutral            |
|       | R1737T     | -1.067        | Neutral            |
|       | S184C      | -0.663        | Neutral            |
|       | K175N      | 0.289         | Neutral            |
| BRCA2 | E2175Q     | -0.536        | Neutral            |
|       | E3342K     | -1.161        | Neutral            |
|       | G500V      | -1.570        | Neutral            |
|       | V1605D     | -5.366        | Deleterious        |
|       | E1571K     | -3.041        | Deleterious        |
|       | I2105V     | -0.132        | Neutral            |
|       | E2903K     | -0.965        | Neutral            |
|       | P606L      | -0.928        | Neutral            |
|       | K3315N     | -0.859        | Neutral            |
|       | S3218F     | -1.141        | Neutral            |
|       | R2861T     | -1.187        | Neutral            |
|       | F312C      | -1.747        | Neutral            |
|       | S3231L     | -1.571        | Neutral            |
|       | D1386N     | -4.722        | Deleterious        |
|       | E3393Q     | -0.493        | Neutral            |
|       | S3123G     | -1.161        | Neutral            |
|       | P1039H     | -6.775        | Deleterious        |
| MLH1  | I262M      | -2.815        | Deleterious        |
|       | A281V      | -2.945        | Deleterious        |
|       | P138R      | -3.415        | Deleterious        |
|       | V647L      | -1.167        | Neutral            |
| MLH2  | G162V      | -8.287        | Deleterious        |
|       | R382C      | -6.674        | Deleterious        |
|       | A107T      | -0.789        | Neutral            |
|       | S142L      | -3.304        | Deleterious        |
|       | A434V      | -1.884        | Neutral            |
|       | Q314R      | -1.502        | Neutral            |
| ATM   | I289M      | -1.232        | Neutral            |
|       | Y1248C     | -5.480        | Deleterious        |
|       | D1053N     | -2.808        | Deleterious        |
|       | I238V      | -0.273        | Neutral            |
|       | I1035V     | 0.447         | Neutral            |
|       | W412C      | -6.890        | Deleterious        |
|       | K2749N     | -2.848        | Deleterious        |
|       | D2997N     | -1.090        | Neutral            |
|       | E2932K     | -3.314        | Deleterious        |
|       | D2988N     | -0.849        | Neutral            |
|       | V1506M     | -0.786        | Neutral            |
|       | E2238Q     | -0.655        | Neutral            |
| Gene    | Alteration | PROVEAN Score | PROVEAN Prediction |
|---------|------------|---------------|--------------------|
| T2333I  | 1.474      | Neutral       |
| T2754A  | -3.037     | Deleterious   |
| H636N   | -1.195     | Neutral       |
| ATR     | Y2637C     | -8.160        | Deleterious        |
|         | W1800R     | -8.567        | Deleterious        |
|         | S1348F     | -2.818        | Deleterious        |
|         | M2266I     | -1.767        | Neutral            |
|         | S61C       | -1.715        | Neutral            |
|         | H1684D     | 0.877         | Neutral            |
|         | E2373V     | -6.183        | Deleterious        |
|         | E560K      | -0.457        | Neutral            |
|         | P2549T     | 0.185         | Neutral            |
|         | G2319E     | -7.600        | Deleterious        |
|         | I693M      | -0.161        | Neutral            |
|         | E54Q       | -0.784        | Neutral            |
|         | N2199S     | -1.026        | Neutral            |
|         | V66M       | -0.518        | Neutral            |
|         | S549C      | -1.204        | Neutral            |
|         | F99L       | -1.518        | Neutral            |
|         | S246F      | -0.880        | Neutral            |
|         | L400F      | -1.426        | Neutral            |
|         | L987I      | -0.402        | Neutral            |
|         | R215S      | -0.670        | Deleterious        |
|         | V1802A     | -2.675        | Deleterious        |
|         | D1243H     | -2.123        | Neutral            |
| MDC 1   | E459K      | -2.178        | Neutral            |
|         | S1253Y     | -2.167        | Neutral            |
|         | S1743C     | -3.572        | Deleterious        |
|         | D1175Y     | -3.250        | Deleterious        |
|         | L122F      | -2.333        | Neutral            |
|         | D1845N     | -1.256        | Neutral            |
|         | S1440F     | -3.550        | Deleterious        |
|         | R114H      | -2.650        | Deleterious        |
|         | D134V      | -3.683        | Deleterious        |
|         | H354Y      | -2.667        | Deleterious        |
|         | N1397I     | -2.783        | Deleterious        |
|         | G1401C     | -1.226        | Neutral            |
|         | H368N      | 3.383         | Deleterious        |
| PARP1   | S274F      | -3.825        | Deleterious        |
|         | E456Q      | -2.167        | Neutral            |
|         | P174T      | -0.649        | Neutral            |
| FANCF   | L86F       | -2.729        | Deleterious        |
|         | A250D      | -2.529        | Deleterious        |

The ATR gene (OMIM 601215) encodes the protein Ataxia telangiectasia and Rad3-related protein, which is a serine-threonine kinase. It senses DNA damage and activates cell cycle checkpoints when induced by endogenous stressors. The protein phosphorylates and activates several other proteins involved in the inhibition of DNA replication and cell division providing time for the cell to repair its DNA, promote recombination and apoptosis [19]. The ATR gene acts along with its closest relative ATM (Ataxia Telangiectasia Mutated). Both the proteins work in consonance and activate the major regulator of stress or damage response gene p53 [20]. The p53 gene is considered to be the “guardian of the genome” and is often inactivated by loss of function mutations in several cancer types over 50%. The frequency of mutations or gene alterations observed in p53 is reported to be high i.e., over 70% in HNSCC [21].
Fig. 3. Box-Whisker plot showing relative expression profile of ATR gene in different grades of HNSC. The X axis denotes the TCGA samples and Y axis denotes the transcripts per million values. The comparison of gene expression patterns between different grades of HNSC returned significant values between normal vs grade 1 ($p=6.4 \times 10^{-4}$), normal vs grade 2 ($p<10^{-12}$), normal vs grade 3 ($p=2.6 \times 10^{-14}$), normal vs grade 4 ($p=1.9 \times 10^{-3}$), grade 1 vs grade 2 ($p=4.5 \times 10^{-4}$), grade 1 vs grade 3 ($p=3.6 \times 10^{-3}$), grade 1 vs grade 4 ($p=4.03 \times 10^{-4}$) and grade 3 vs grade 4 ($p=1.56 \times 10^{-2}$). A p value less than 0.05 is considered to be significant.

Fig. 4. Kaplan–Meier plots showing the association of high level expression of ATR gene in male and female HNSC patient’s survival. The x-axis represents time in days and y-axis shows the survival probability. The blue line indicates high level expression in female patients and the red line indicates high level expression in male patients. A high level expression of the ATR gene presented with a low survival rate in female patients ($p$ value $<0.0001$)
The ATR gene mutations have been reported in melanoma, lung, stomach, bladder, uterine and endometrial cancers with less than 2% alteration in HNSCC. The present study identified less than 5% mutations in the ATR gene. Although germline mutations in ATR gene are rare events, such mutations have been identified in patients with Seckel syndrome, who present with an autosomal dominant type of inheritance [22]. A study reported novel mutations in ATR gene in oropharyngeal squamous cell carcinoma (OpSCC) patients, with an HPV negative status. They concluded that the mutations identified could lead to functional loss of the gene activity thereby indicating the important role of the gene in the etiology of OpSCC [23]. Another study by Parikh et al., demonstrated the upregulation of ATR-CHEK1 pathway in OSCC cell lines with the loss of ATM. They observed gene amplification, translocations and gain of copy number in ATR gene. The qPCR assay also showed overexpression of ATR in OSCC cell lines. The inhibition of ATR with siRNA was found to increase the sensitivity of OSCC cells to ionizing radiations [24]. A very recent study by Farah and colleagues, reported the association of FA/BRCA of the double stranded break pathway (DSB) with the malignant transformation of oral leukoplakia to oral squamous cell carcinoma (OSCC) [25].

The ATR inhibitors have been well documented using in vitro approaches. A recent study demonstrated the sensitisation of HNSCC cell lines to cisplatin upon inhibition of ATR with AZD6738. The results were also replicated successfully in patient-derived xenograft tumors. The HPV negative and HPV positive cell lines were also found to be sensitive to cisplatin by inhibition of ATR [26]. A critical determinant of sensitivity to radiotherapy in patients with oropharyngeal cancer with HPV positive patients has been linked to defects in signal mediators and repair genes. The principal target proteins were PARP; DNA-Pk, ATM and ATR. Also inhibitors of proteins involved in cell cycle checkpoint activation were also tested for radiosensitization of HNSCC [27]. Another interesting finding about the role of ATM and ATR in relation to HPV DNA replication was recently documented. The inhibition of ATR pathway resulted in the suppression of HPV amplification and maintenance of the genome. The underlying mechanisms could be through molecular pathways such as transcripational regulation and cell cycle checkpoints [28,29]. Thus, the present study provides preliminary details on the genetic alterations in ATR-ATM pathway and its possible involvement in the development of HNSCC. The in silico approaches have been found to be successful in screening for alterations in candidate gene family in association with HNSCC [30]. DNA damage response gene expression levels significantly correlated with the tumor grades in HNSCC patients. The defects in cell cycle checkpoints of DNA repair genes, such as mutation or abnormalities, may lead to the wide spectrum of human diseases.

4. CONCLUSION

The present study thus highlights the mutations in DNA damage response genes which may have a significant role to play in HNSCC. Further experimental validations are warranted to derive an association between the preliminary data obtained and the disease phenotype.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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