DECODING THE GENETIC ALTERATION IN GENES OF PARP FAMILY AND THE POSSIBLE ASSOCIATION WITH HNSCC

Running title: PARP gene family and its association with HNSCC

ABSTRACT

INTRODUCTION: Genetic alterations have long been associated with the transformation of normal cells into malignant cells. Several genes are related to exhibiting the phenotype. The *PARP* gene family is mainly involved in maintaining genome stability. They play an important role in DNA repair and the programmed cell death process.

AIM: To analyse the genetic alteration in PARP family and to determine its association with HNSCC

MATERIALS AND METHOD:

Cbioportal was used as the primary database for identifying the mutations and variations. The data generated in the form of oncoprint was further assessed for frequency of occurrence, type and novelty.

RESULTS & DISCUSSION: It can be observed that greater amplification was found in the *TIPARP* gene which is 14% among all the 17 genes of the family. Also to add on, *PARP 14* and *PARP 15* show amplification patterns in similar groups of patients. Several types of mutations such as truncated, splicing deep deletion were found in most of the genes. The *TIPARP* gene was up-regulated in HNSCC patients. The Caucasians experiencing low/medium expression of *TIPARP* showed greater rates of survival than highly expressed African Americans. Similarly, males presenting with low or medium expression of TIPARP showed a greater rate of survival than the highly expressed females.

CONCLUSION: *TIPARP* could be a promising prognostic marker for screening populations vulnerable to acquiring HNSCC.

Keywords: HNSCC, PARP gene family, genetic alteration, gene expression, novel variants, polymorphism, innovative techniques and innovative technologies.

INTRODUCTION:

Head and neck squamous cell carcinoma (HNSCC) which is the major type of cancer that is most common world wide. Mutation in the TP53 gene sequence which is the somatic genomic alteration that potentially gives rise to HNSCC. Several other gene mutations have also been implicated in the development of oral cancer. The treatment procedure involves surgery, chemotherapy, radiotherapy etc. (1). HNSCC occurs majorly in 5 anatomical sites which consist of oral cavity, oropharynx, Nasopharynx, hypopharynx and larynx. HNSCC is the cancer that can be cured if it is detected early and often there won't be any symptoms visible, hence it can be avoided at the earliest and detected only when it becomes severe (2). Tobacco smoking is the primary reason for HNSCC and it is mainly seen in males rather than females. The detection and the diagnosis involves immunohistochemistry, PCR, in situ hybridisation (3).

Poly (ADP-ribose) polymerases (PARPs) are a family of enzymes that exhibit the ability to catalyze the transfer of ADP-ribose to target proteins. Cellular processes, transcription, replication, recombination and DNA repair are a few pathways to mention where PARPs play a vital role. With a special emphasis on the involvement of PARP proteins in DNA repair is of great interest, because certain transformed cells principally rely on PARP mediated DNA repair for survival. Several reports on PARP inhibitors have been shown to increase tumor sensitivity to DNA-damaging agents (3,4). It is seen that among *PARP, PARP1* and *PARP2* has a catalytic activity and is useful when there is DNA breakage (5). Genetic alteration has a different pathway

and is seen when there is high DNA damage and it is also the multistep accumulation in the genomic landscapes which develops into HNSCC due to overexpression of oncogenes, silenced tumor suppressor (6).

Numerous in silico methods have been used to identify potential variations or mutations in the genome, which could act as potential drivers in triggering disease phenotypes. In the study conducted by Aparna et al, it was seen that matrix metalloproteinases and their association in HNSCC since *MMP* are involved in malignant transformation of a tumor and studied the expression of *MMP* in HNSCC patients (7). The PARP inhibitors have been found to be useful in HNSCC treatment and the study conducted by Wurtser showed the association of the *PARP* gene family with HNSCC (8). Based on the previous research it can be seen that there was very little study on the *PARP* gene family and also negligible research done on its association with HNSCC.Our team has extensive knowledge and research experience that has translate into high quality publications(9–20),(21–25). (26) (27) (28). This research aims to decode the genetic alteration in the genes of the *PARP* gene family and their association with HNSCC.

MATERIALS AND METHOD:

Data source

It is a retrospective study and the patient's data has been derived from cBioportal (29) which contains all the patient's details obtained from different cohorts. Information about the genetic alterations throughout the genomic landscape of HNSCC patients are deposited in the repository (30). The complete profiling of each case in the data set and the demographic details are given in table 1. Genes used in this study were *PARP1, PARP2, PARP3, PARP4, TNKS, TNKS2, PARP6, TIPARP, PARP8, PARP9, PARP10, PARP11, PARP12, ZC3HAV1, PARP14, PARP15, PARP16*. The genes were queried among the HNSCC dataset and the results were used for further analysis.

Oncoprint data analysis

The information obtained includes the allele frequency, variation, protein coding, amino acid, deletion, insertion etc. the putative association involving the variations, genome, novel variation and the disease phenotype.(29,31)

gnomAD data analysis

This type of investigation involves the large scale sequencing projects and the dataset containing unrelated sequences and public release and compares the variants documented and reported gnomAD repository (29,31).

Gene expression and survival analysis:

The expression of the gene presenting with highest frequency of gene alteration in HNSCC was analysed using the UALCAN (http://ualcan.path.uab.edu/cgi-bin/TCGA-survival) database. Survival curve analysis based on the tumor grade and expression profile was performed to demonstrate the putative role of *PARP* family of genes with HNSCC. Combined survival effect analysis of gene expression and other clinical parameters such as race, gender, tumor grade, cancer subtypes were assessed using a log-rank test that generated a p value which was further used to indicate statistical significance of survival correlation between groups. The test that was used is log rank test (32).

Comment [SK1]: 1: it's repetitive. 2: Report the significant value: A p value less than 0.05 was considered to be significant

RESULTS:

cBioportal database was the primary source to obtain the information of patients with head and neck squamous cell carcinoma. The table 1 shows the demographic details of the patients and the age group of patients was between 19-90 years. Table 2 shows the gene alteration in PARP family and it contains total of 17 genes including *PARP1, PARP2, PARP3, PARP4,TNKS, TNKS2, PARP6, TIPARP, PARP8, PARP9, PARP10, PARP11, PARP12, ZC3HAV1, PARP14, PARP15, PARP16*. Among these genes it was found that *TIPARP* showed 14% of genetic alterations and which is greatest. The *PARP8* gene contains 16 gene alterations and is highest on comparing all the 17 genes. *PARP1, PARP2, PARP6, TIPARP, PARP9, PARP10, PARP11, PARP14, PARP15* shows amplification of genes, *PARP3, PARP4* shows deep deletion. *TNKS, TNKS2, PARP8, PARP12, ZC3HAV1* have both amplification and deep deletion. Under *PARP2*, the N129K gene shows an already existing mutation. Under *PARP4*, E216Q, P120L, EL067K; under TNKS, R245C, V697M, S132F; under *PARP8*, R488H; under *PARP9*, R617Q; under *PARP10*, R753C, ZC3HAV1, R455T, I574V; under *PARP14*, P988L shows already existing mutation others contain novel mutation.

TIPARP showed higher frequency of gene amplification, *TNKS* showed more deep deletion. *PARP4*, *TNKS*, *PARP8*,*PARP11*, *PARP12*, *ZC3HAV1*, *PARP14*, *PARP16* showed truncating mutations. *PARP2* showed inframe mutation. *PARP6*, *PARP8* showed splice-site mutation. Except *PARP16*, all the other genes had missense mutations. *PARP12* and *ZC3HAV1* showed amplification and deep deletion in the same patients. *PARP14 & PARP15* showed the same pattern amplification in the same patients.

The expression of *TIPARP* was upregulated in HNSCC individuals in comparison to normal individuals. The p value was found to be 1.82×10^{-1} which was found to be insignificant (Figure 2). Upon analysing survival probability based on the Kaplan Meier analysis of *TIPARP* gene expression classified based on race, it was found that low/medium expression in caucasian individuals showed maximum survival rate when compared to the high expression African-American, the p value was found to be $p = 0.045$ (Figure 3a). The Kaplan Meier analysis of *TIPARP* expression level classified based on gender showed that low/medium expression male have greater survival rate when compared to high expression females and the p value was found to be $p = 0.027$ (Figure 3b).

Table 1: showing the demographic details of patients analyzed 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 Data not available: 11

Comment [SK2]: Please represent better the table 1.

Comment [SK3]: Mean value is request.

Comment [SK4]: Report the percentages in all value.

Neoplasm Histologic grade	Grade 1: 63 Grade 2: 311 Grade 3: 125 Grade 4: 7 Grade GX: 18 Data not available: 4
Race category	White: 452 African: 48 Asian: 11 American Indian or Alaska native: 2 Data not available: 15

Table 2: showing the gene alteration in the PARP family of genes.

Gene	Protein encoded	Cytogenetic loci	% of genetic alterations	Gene alterations	Variant allele frequency	gnomAD frequency
PARP1	Poly(ADP-ribose) polymerase 1	1q42.12	2	Amplification S274F P881L F586L E456Q P174T	 0.23 0.04 0.01 0.27 0.54	 Novel Novel Novel Novel Novel
PARP2	Poly(ADP-ribose) polymerase 2	14q11.2	2.4	Amplification A541dup M432I N129K	 0.26 0.10 0.16	 Novel Novel rs139090502
PARP3	Poly(ADP-ribose) polymerase family member 3	3p21.2	1.2	Deep deletion E277D R472Q	 0.67 0.11	 Novel Novel

PARP4	Poly(ADP-ribose) polymerase family member 4	13q12.12	2.6	Deep deletion E216Q A637T H803Q D952N W1573R P120L E1067K P1336S Q174*	 0.72 0.16 0.22 0.24 0.19 0.31 0.06 0.13 0.13	 rs145170390 Novel Novel Novel Novel rs199585627 rs372126761 Novel Novel
TNKS	Tankyrase	8p23.1	5	Amplification Deep deletion E441K G1013C R245C V697M N555KFS*2 S1264N S132F	 0.23 0.28 0.26 0.85 0.15 0.43 0.18	 Novel Novel rs773491393 rs1043487769 Novel Novel rs774407820
TNKS2	Tankyrase 2	10q23.32	1.8	Amplification Deep deletion G677D N271S H597N V246E A1062V A219V	 0.05 0.16 0.23 0.52 0.29 0.45	 Novel Novel Novel Novel Novel Novel
PARP6	Poly(ADP-ribose) polymerase family member 6	15q23	0.6	Amplification I213V E568=	 0.37 0.63	 Novel Novel
TIPARP	TCDD inducible poly(ADP-ribose) polymerase	3q25.31	14	Amplification G239E H354Y	 0.15 0.07	 Novel Novel

PARP8	Poly(ADP-ribose) polymerase family member 8	5q11.1	10	Amplification Deep deletion S761G R416T H426Y I183S X476_splice R488H R616K S468C R88K E443Q E532Q Y581F Q556* F340L	 0.79 0.14 0.12 0.26 0.16 0.29 0.16 0.08 0.16 0.25 0.47 0.18 0.19 0.03	 Novel Novel Novel Novel Novel rs1421801606 Novel Novel Novel Novel Novel Novel Novel Novel
PARP9	Poly(ADP-ribose) polymerase family member 9	3q21.1	5	Amplification H408Q G17C E824Q R617Q	 0.26 0.49 0.57 0.51	 Novel Novel Novel rs559272508
PARP10	Poly(ADP-ribose) polymerase family member 10	8q24.3	12	Amplification F906L R753C A781S P98S	 0.16 0.22 0.23 0.24	 Novel rs139166854 Novel Novel
PARP11	Poly(ADP-ribose) polymerase family member 11	12p13.32	4	Amplification T160M T29K R296*	 0.21 0.37 0.32	 Novel Novel Novel

PARP12	Poly(ADP-ribose) polymerase family member 12	7q34	2.6	Amplification Deep deletion W381C Q157* R531* K428R C195R G19Afs*16	 0.36 0.28 0.11 0.30 0.27 0.33	 Novel Novel Novel Novel Novel Novel
ZC3HAV1	Zinc finger CCCH-type containing, antiviral 1	7q34	2.4	Amplification Deep deletion R455T L126H I574V Q255*	 0.37 0.70 0.27 0.07	 rs1403439859 Novel rs150148096 Novel

PARP14	Poly(ADP-ribose) polymerase family member 14	3q21.1	7	Amplification N401Kfs*4 S1754L T1566Nfs*3 I1515M P988L Q465K I172V G5D E964K G1135E D189H Q1276* N1099Ifs*17 G1499E	 0.31 0.20 0.14 0.08 0.19 0.53 0.18 0.23 0.20 0.21 0.18 0.15 0.11 0.17	 Novel Novel Novel Novel rs771490414 Novel Novel Novel Novel Novel Novel Novel Novel Novel
PARP15	Poly(ADP-ribose) polymerase family member 15	3q21.1	5	Amplification Q357H P438T	 0.63 0.44	 Novel Novel
PARP16	Poly(ADP-ribose) polymerase family member 16	15q22.31	0.2	W200*	0.20	Novel

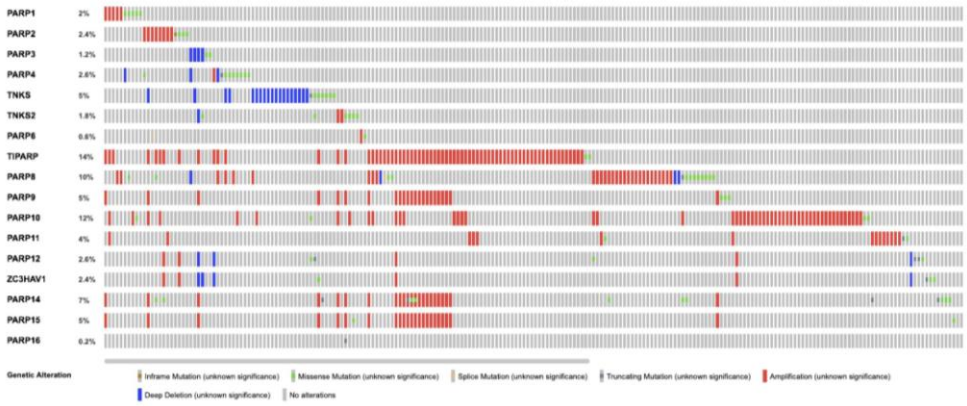

Figure 1: showing the oncoprint data that is demonstrating the alterations in the PARP gene family in the HNSCC patients

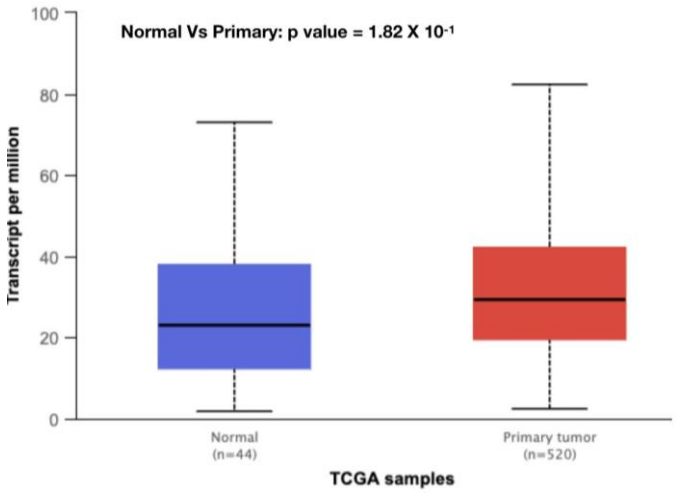

Figure 2: Box-Whisker plot showing relative expression profile of *TIPARP* gene (Normal vs primary tumor). The X axis denotes the TCGA samples (blue bar indicates normal and red bar indicates primary tumor) and Y axis denotes the transcripts per million values. The comparison of gene expression patterns between normal vs primary tumor was insignificant ($p = 1.82 \times 10^{-1}$). A p value less than 0.05 was considered to be significant.

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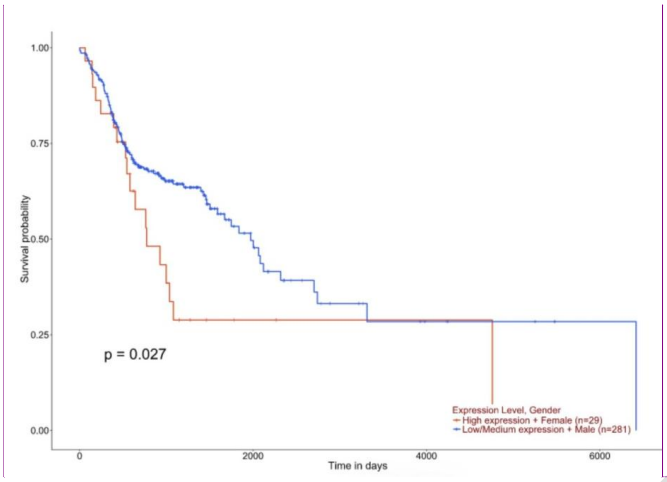

Comment [SK6]: Please can express the time in months?

Figure.3 (a) : Kaplan Meier plot showing the effect of *TIPARP* expression level classified based on gender of HNSCC patients. The x-axis represents the time in days and the y-axis represents the survival probability. The blue line indicates low expression of *TIPARP* in males and the red line indicates high expression in females. A significant difference in the level of gene expression between the two groups was observed ($p=0.027$); $p<0.05$- significant.

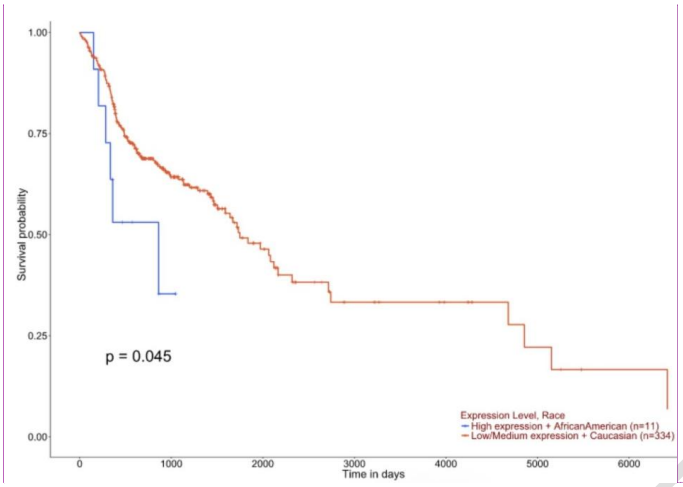

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Figure.3 (b) : Kaplan Meier plot showing the effect of *TIPARP* expression level classified based on race of HNSCC patients. The x-axis represents the time in days and the y-axis represents the survival probability. The red line indicates low/medium expression of *TIPARP* in Caucasians and the blue line indicates high expression in African American population. A significant difference in the level of gene expression between the two groups was observed (p=0.045); p<0.05-significant.

DISCUSSION

Head and neck squamous cell carcinoma is the most common type of cancer which is diagnosed every year (33). The study is done to understand the alterations that were observed in the *PARP* gene family and their involvement in HNSCC. This study provides us with information that is already not available and usage of data sources to easily obtain information about patients and perform basic research to accumulate preliminary data. Genetic alteration is a very time consuming procedure when done manually and expensive too. PARP plays an important role in DNA repair pathways (Vyas *et al.*, 2013), with a special emphasis on base excision repair (BER), which is involved in DNA repair of single strand breaks (SSBs). Since in most of the cancer types BER is impaired eventually leading to inhibition of poly(ADP-ribose) polymerase (PARP). This results in the conversion of SSBs to double strand breaks (DSBs).

The expression of *PARP1* is increased in oral squamous cell carcinoma. The expression of PARP was seen at subcellular level. The over-expression in premalignant tumors also paved the way for diagnosed OSCC in the future (34). According to the study conducted by Maria *et al*, it was found that the expression of PROX1 gene was found to be expressed as tumor suppressor gene (35). A study conducted by Gesche indicated that XIAP is involved in the oral squamous cell carcinoma and also the Kaplan Meier curve indicated the XIAP association in unfavourable prognosis of oral squamous cell carcinoma and other curve showing the survival rate that was insignificant (35,36). The study that was conducted by Yao *et al*, found that usage of microRNAs in association with OSCC and observed that fibroblast transfers microRNA to oral squamous cell carcinoma cells. Overexpression of miR-34a-5p could lead to tumorigenesis and contribute to the aggressiveness of the cells (37). Usage of Rab5a was seen in many different types of cancer. A study conducted by Dizhang et al. showed that in 49.3% of OSCC patients Rab5a was overexpressed (37,38). The gene alteration studies on various genes have also been done for HNSCC and other cancers as well (39),(40),(41),(42),(42,43),(44),(45)**.**

CONCLUSION

The present study brings in a conclusion that *TIPARP* could be considered as a prognostic marker in the case of HNSCC. Although the gene expression pattern between normal and tumor tissues do not produce a significant variation, the expression level in different races and genders contributed to significant change in the survival of HNSCC patients. More clinical studies have to be carried out to derive an association between *TIPARP* and HNSCC.

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