RESEARCH ARTICLE

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# Overexpression of *XRCC1* is Associated with Poor Survival in Patients with Head and Neck Squamous Carcinoma and Has Potential to Be Used as Targeted Therapy by Synthetic Lethality

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## Abstract

**Background:** Head neck squamous cell carcinoma (HNSC) is globally prevalent cancer attributed to tobacco habit. Despite the significant advances in early diagnosis and treatment of HNSC chemo-radio resistance are routinely observed in patients. Aberrant DNA repair mechanisms mainly microhomology mediated DNA end joining (MMEJ) pathway causing deleterious mutations and is implicated in treatment resistance. X-ray cross complimenting group 1 (*XRCC1*) has recently been shown to play an essential role in MMEJ making *XRCC1* a potential therapeutic target to render tumors chemo-radiosensitive. This study analyzes the correlation between the expression level of *XRCC1* gene with survival, regulation by miRNA and synthetic lethality partners in HNSCC. **Materials and Methods:** *XRCC1* gene expression was evaluated in 520 HNSC patients and 44 of normal tissues using the UALCAN (TCGA) database and its correlation with survival outcome of HNSC patients was analyzed by Kaplan-Meier plot. Infiltration of immune cells in tumors was analyzed by "Tumor-Infiltrating Immune Estimation Resource (TIMER) and promoter methylation status of *XRCC1* in samples was analysed by UALCAN. STRING was used to find gene interacting partners of *XRCC1*. **Results:** *XRCC1* was significantly overexpressed in primary tumor of HNSCC and significantly increased with tumor stages and grade and associated with poor survival rate. High *XRCC1* expression in HNSC was positively correlated with infiltration level of B cells naïsve, CD4+ and macrophages. **Conclusion:** These results indicate that *XRCC1* is a prognostic marker for predicting survival in HNSC patients. Understanding how *XRCC1* leads to treatment resistance and modulate immune response can lead to development of targeted therapy.

**Keywords:** HNSCC- *XRCC1*- MMEJ- Prognostic biomarker- UALCAN (TCGA) database

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## Introduction

Head neck squamous cell carcinoma (HNSC) is globally prevalent cancers and common in Indian men and is attributed to tobacco use (Jethwa and Khariwala, 2017). Despite the significant advances in early diagnosis and treatment of HNSC by surgery and concurrent radio-chemotherapy is routinely observed in patients leading to recurrence, morbidity and mortality (Kim, 2017). Mutagenic agents including ionizing radiation can trigger the accumulation of genetic alteration resulting it affect the cell cycle regulation, proliferation, apoptosis and modification in DNA repair. DNA repair mechanism plays an important role in the protection of the genome against carcinogenic agents and preventing the cancer cells altered from continuing the cell cycle and their inappropriate proliferation. DNA repair genes are the key regulators in different metabolic pathways and maintain the structural integrity and functions of DNA. X-ray repair cross-complementing 1 (*XRCC1*) is the first human gene identified that affects cell sensitivity to ionizing radiation (London, 2015). *XRCC1* participates in base excision repair (BER), single-strand break repair (SSBR) and microhomology mediated DNA end joining (MMEJ) to repair the DNA damage induced by ionizing radiation, alkylation and chemical mutagens. Aberrant DNA repair mechanisms causing deleterious mutations are implicated in treatment resistance (Sfeir, 2015; Sharma et al., 2015). Hence biomarkers to assess DNA repair in tumors is warranted for tailoring therapeutic regimen and better prognosis.

*XRCC1* (Figure 1) has recently been shown to play an

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essential role in DNA repair mechanism mainly MMEJ, making *XRCC1* a potential therapeutic target to render tumor chemo-radiosensitive (Sfeir, 2015; Dutta et al., 2017; Ali et al., 2020). The objective of this study was to analyzes the correlation between the expression level of *XRCC1* gene with tumor grade, tumor immunity and survival in HNSC using bioinformatics approach.

## Materials and Methods

*Correlation between XRCC1 expression and survival analysis*

Gene expression data of 520 HNSC tissues and 44 of normal tissues was downloaded from TGCA RNAseq (UALCAN) publically open database in Jane 2022 (Chandrashekar et al., 2017) and analysed, moreover, methylation of *XRCC1* gene was also analysed TCGA tissue samples. We downloaded survival time information from TCGA (including Overall survival and Disease-free survival). These tumor samples were divided into high and low expression group according the median *XRCC1* expression value. The survival analysis was done using the Kaplan-Meier plot method (Győrffy et al., 2013) the significant differences determined by a long rank test. P< 0.05 considered as statistically significant for overall survival.

*Correlation of the XRCC1 expression with clinical parameters in HNSC*

A correlation analysis of the expression levels of *XRCC1* with the different clinical stages of cancer (HNSC: stage I, n = 27; stage II, n = 71; stage III, n = 81; stage IV, n = 264) and gender of patients (HNSC: Female, n = 136; Male, n = 383) was performed using the TCGA database. Spearman correlation test was used to analyze the correlation between *XRCC1* expression and tumor stages, box plots obtained from TCGA RNAseq database. Then, the correlation between the expression levels of *XRCC1* and the gender of patients was analyzed using the Wilcoxon test to identify the significant differences between the two groups, box plots obtained from TCGA RNAseq database (Chandrashekar et al., 2017).

*Methylation of XRCC1 with survival of HNSC*

Methylation status of *XRCC1* gene was analysed using SurvivalMeth publically available database (Zhang et al., 2021). This tool provides comparative analysis of gene methylation level with patient's survival.

*XRCC1 expression and immune cell infiltration*

For the investigation of tumor immune cells in the RNAseq TGCA database, the Tumor Immune Estimate Resource (TIMER) is a freely accessible computational tool (Li et al., 2017). Based on the gene expression profile, a deconvolution method is employed to determine the amount of tumor immune infiltration cells. Eight different types of tumor immune infiltration cells (B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils, and DCs) were chosen for our investigation, which examined the relationships between the expressions of the *XRCC1* gene and TIICs in patients with HNSC. For measuring the Spearman's correlation, tumor purity was taken into account (Li et al., 2017). Due to the expected negative relationships between highly expressed genes in the invading immune cells and tumor purity. Statistical significance was defined as a p value 0.05.

*Gene-function interaction analysis of XRCC1*

Virtual screening of *XRCC1* associated genes were performed using STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) a publically available database (Szklarczyk et al., 2019; Szklarczyk et al., 2021). It is user friendly tool provide hypothetic gene function and functional essays. This tool also predict the function of given query genes, the interactions of genes are in the form of physical, co-expression, prediction, proteins homology, co-localization, genetic and pathway interaction (Szklarczyk et al., 2021).

*miRNA regulating XRCC1 expression*

The publically available database miRWalk was utilised to find *XRCC1* associated miRNAs in humans with predicted and validated miRNA binding sites. The miRNA target site prediction for miRWalk is based on the random-forest approach. The list of all miRNAs associated with *XRCC1* displayed on \were arranged as per their expression levels if upregulated or downregulated. The top three upregulated miRNAs were further tested for expression level in HNSC using the publically available mirCancer database (Xie et al., 2013).

*Correlation with XRCC1 expression and miR-21*

Expression of miR-21 and survival information was obtained from TCGA miRNA database. MiRNAs control the gene expression by suppressing the gene expression. In this study miRNA-21 was over expressed in TCGA samples.

*Mutation analysis in XRCC1 and miRNA-21*

Somatic mutations in *XRCC1* gene were analyzed using the publically COSMIC database (Forbes et al., 2015). The mutations in the miRNA-21 binding site were identified using the database miRNASNP-V3 Liu et al., 2021). Gene and miRNA binding site were predicted by miRWalk database (Stich et al., 2018).

*Statistical analysis*

Bioinformatics statistical analysis was performed by the online freely available 'R' software. Student t-test was used for analysis of expression differences into normal and tumor tissues of TCGA samples. Logrank test was used for comparison of K-M survival curve and genetic alteration prognostic plots. Moreover, P value <0.05 was considered as statistical significant difference.

## Results

*XRCC1 is significantly overexpressed in HNSC*

Analysis of the *XRCC1* expression level in tumor samples (n = 520) and normal tissue samples (n = 44) indicated that *XRCC1* expression level was significantly higher in HNSC tissues than in the normal tissues (P <

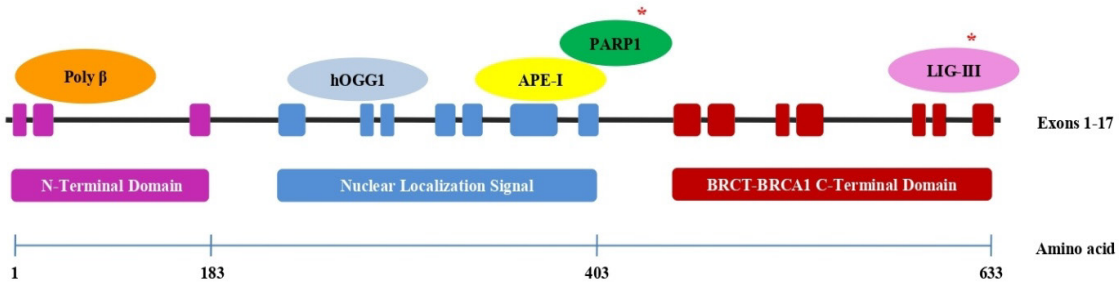


Figure 1. *XRCC1* Gene Overview and Binding Sites for Other Genes (* indicates proteins of MMEJ repair pathway)

Table 1. Methylation Site Differences of XRCCs in HNSC and Normal Tissues

| Gene | Site | Average of tumor | Average of Normal | Delta value | P value |
|---|---|---|---|---|---|
| *XRCC1* | cg01880404 | 0.049194 | 0.060121 | -0.01092 | 6.82E-08 |
| | cg08112313 | 0.277669 | 0.230684 | 0.046984 | 0.000145 |
| | cg15167433 | 0.042735 | 0.051365 | -0.00862 | 8.24E-05 |

0.001) (Figure 2). The higher mRNA expression level of *XRCC1* was significantly associated with poor over all patient survival and disease free OS in patients with HNSC (Figure 2D-E).

*Correlation of the expression levels of XRCC1with the clinical parameters of HNSC*

Statistically significant differences were observed in the patients with HNSC in the *XRCC1* expression ($P < 0.05$), with a positive correlation between the tumor

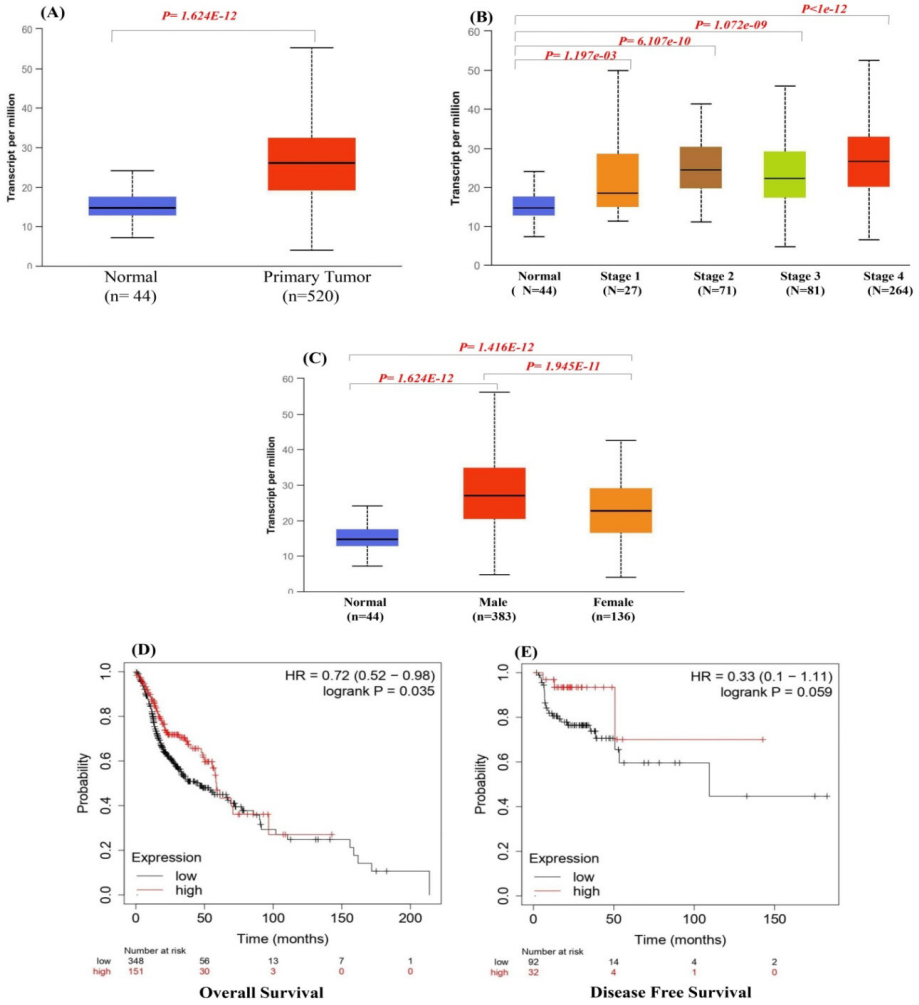


Figure 2. (A) Expression of XRCC1 in TCGA samples, Correlation of XRCC1 with clinical factors (B) Correlation between XRCCs expression and tumor stage in HNSC patients (C) Correlation between XRCC1 expression and gender in HNSC patients, (D-E) Overall and Disease Free Survival

Table 2. Synthetic Lethality Partner of *XRCC1*

| S N | Synthetic Lethality Partner | Expression | Type of Cancer | Reference |
|---|---|---|---|---|
| 1 | *PARP1* | Up regulate | Ovarian Cancer | [7] |
| 2 | *LIG1* | Up regulate | Ovarian Cancer | [40] |
| 3 | *BRCA2* | Down regulated | In vitro | [22] |
| 4 | *ATM* | Up regulate | Breast Cancer | [40] |
| 5 | *ATR* | Up regulate | Breast Cancer | [41] |
| 6 | *Wee1* | Up regulate | Breast Cancer | [40] |
| 7 | *MRE11* | Up regulate | Oral Cancer | [42] |

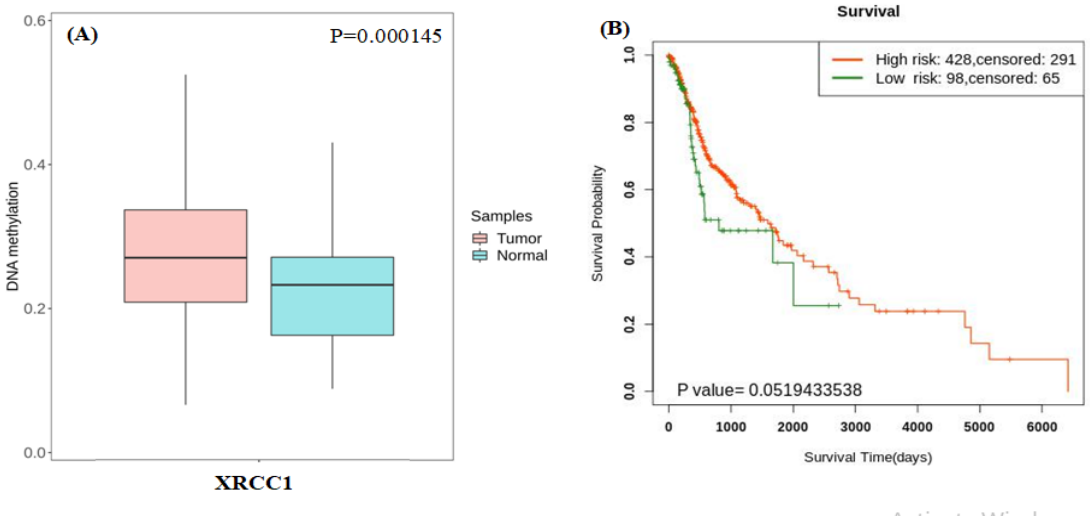


Figure 3. DNA-Methylation of *XRCC1* Gene

*miRNA regulating XRCC1 expression*

The miRWalk database search revealed more than twenty five hundreds miRNAs that were associated with *XRCC1* which are intact on different position of gene according to the result generated by miRWalk database. We further screen out these miRNAs using mirCancer database, out of 2609 miRNAs only 63 were up/down regulated in HNSC, thus mirCancer database provided the expression profile of cancer related miRNAs which were studied in clinical samples. We were filtered these miRNAs and most over expressed miRNAs i.e. has-mir-21 were select for further analysis.

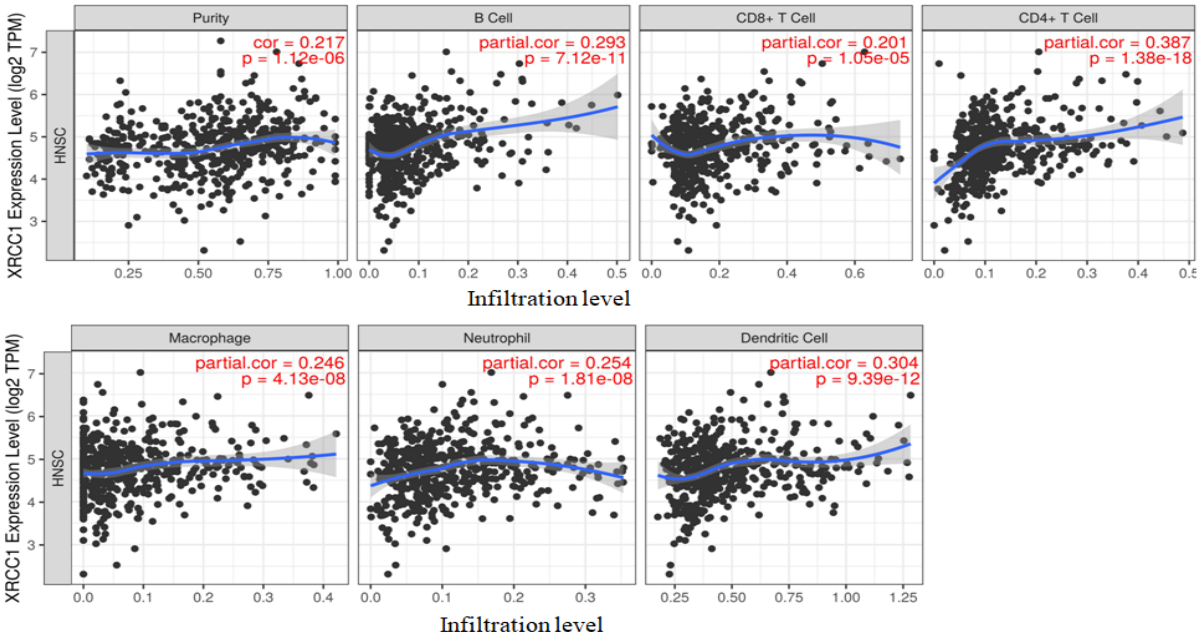


Figure 4. Tumor Purity and Immune Cell Infiltration Associated with *XRCC1* Expression in Patients with HNSC

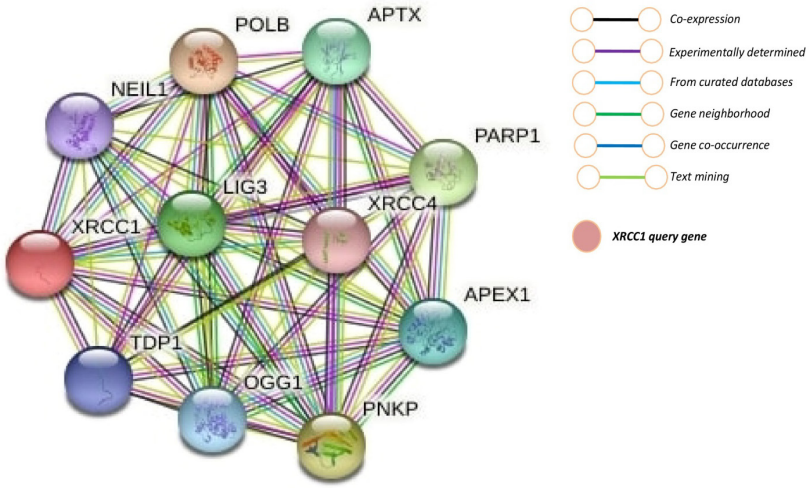


Figure 5. Gene Interaction Network Associate with *XRCC1* Function

Table 3. Overall Survivals of MMEJ Genes of TCGA RNAseq HNC Samples

| S N | MMEJ Genes | Expression | Hazard ratio (HR) | Logrank P value |
|---|---|---|---|---|
| 1 | *XRCC1* | Overexpress | 0.72 (0.52- 0.98) | 0.035* |
| 2 | *LIG1* | Overexpress | 0.67 (0.48-0.92) | 0.013* |
| 3 | *POLQ* | Overexpress | 0.71 (0.52-0.95) | 0.022* |
| 4 | *FEN1* | Overexpress | 1.29 (0.98-1.69) | 0.065 |
| 5 | *LIG3* | Overexpress | 0.80 (0.61-1.04) | 0.099 |
| 6 | *PARP1* | Overexpress | 0.78 (0.57-1.05) | 0.100 |
| 7 | *MRE11* | Overexpress | 0.75 (0.56-1.00) | 0.052 |
| 8 | *POLB* | No Significant expression | 0.74 (0.57-0.97) | 0.029* |
| 9 | *RAD50* | No Significant expression | 1.32 (0.99-1.75) | 0.060 |

stage and gene expression (Figure 2B). The highest gene expression was observed in stage IV HNSC (Figure 2C). In addition, mRNA levels of *XRCC1*in HNSC patients were higher in men than in women ($P < 0.05$; Figure 2B-C). Overall, these findings imply that the expression levels of *XRCC1* are partially correlated with the clinical parameters in HNSC patients.

*Methylation of XRCC1 with survival of HNSC*

Based on our finding suggesting that *XRCC1* overepression can be used as potential independent risk factor for patients with HNSC, we further analyzed the methylation sites of *XRCC1* using Survival Meth (Li et al., 2019). For Comparison of the methylation levels in HNSC tumor tissues and in normal tissues (Table 1). Then, we divided the samples with differentially methylated sites

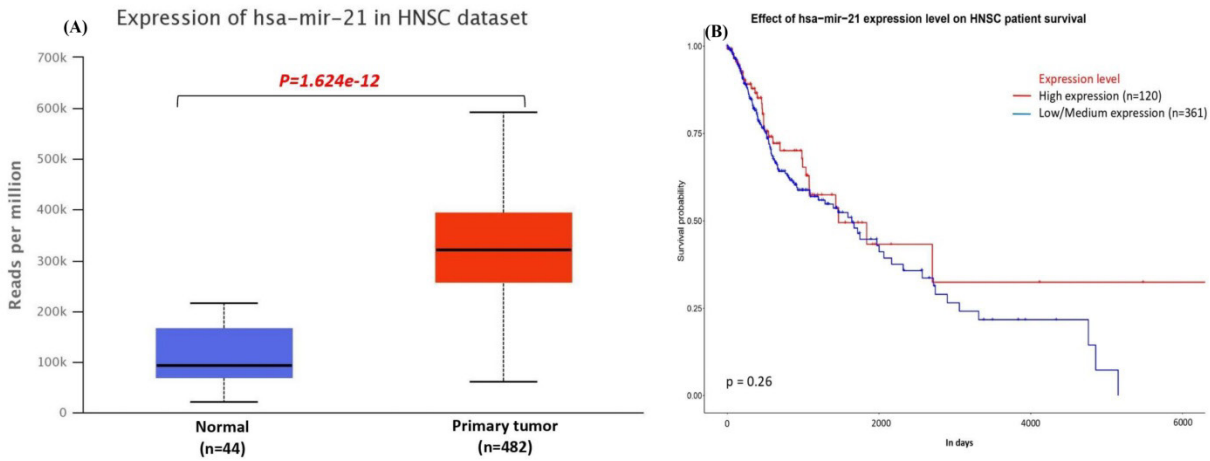


Figure 6. (A) expression of miR-21 in HNSC (B) effect of miR-21 expression on HNSC patients survival

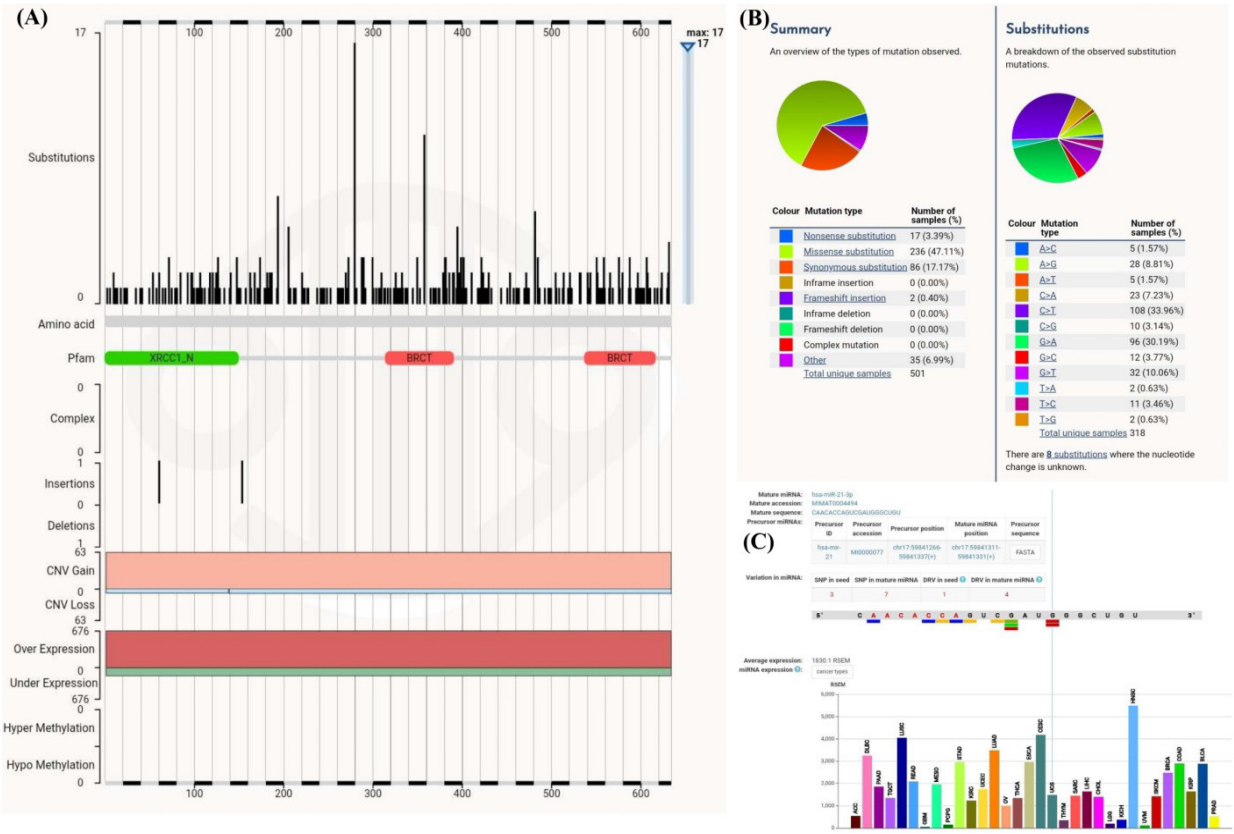


Figure 7. (A-B) mutations in *XRCC1* coding region, (C) mutations in mature miRNA-21

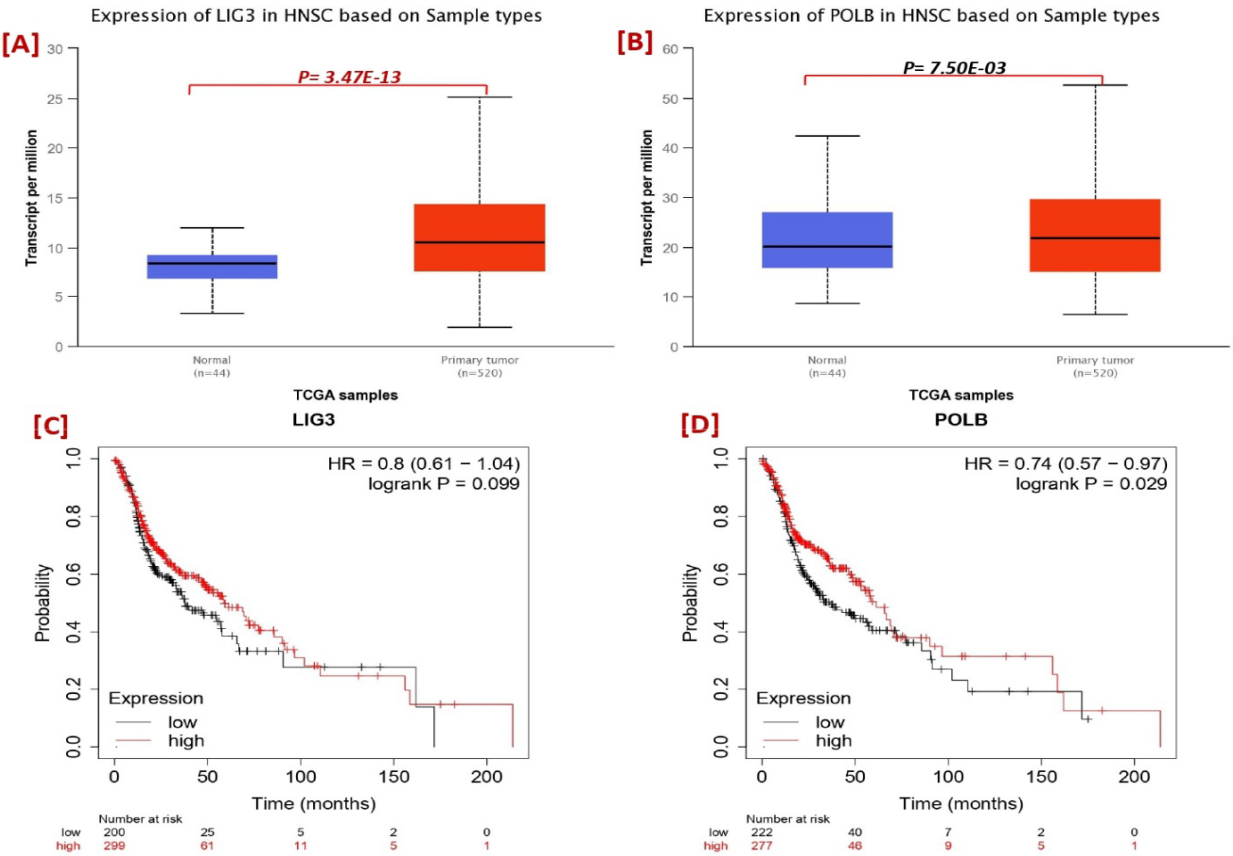


Figure 8. Expression and Survival Analysis of *LIG3* and POLB

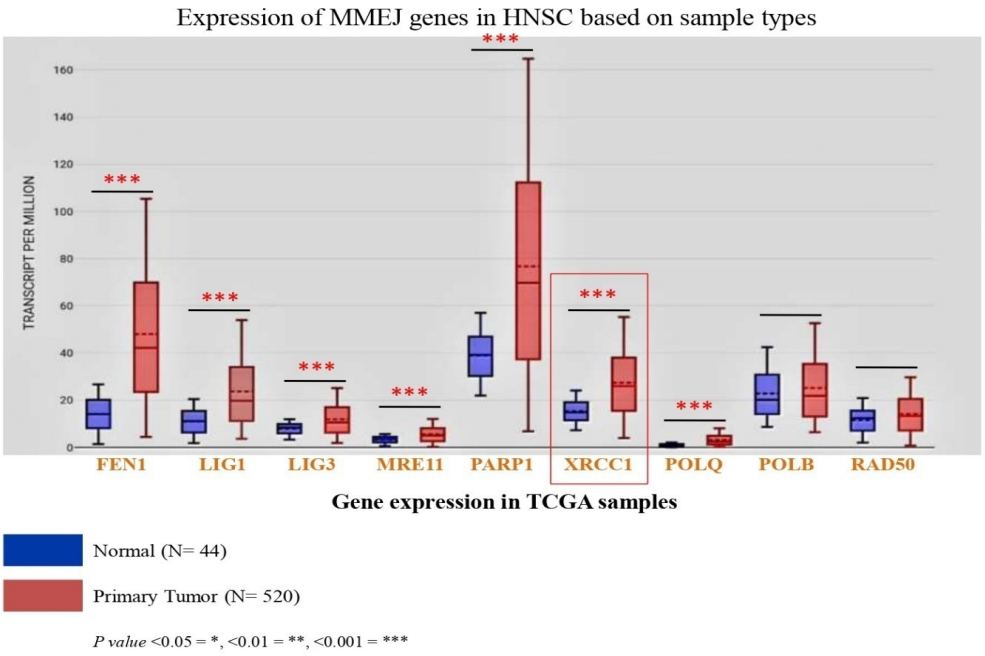


Figure 9. Expression of MMEJ Pathway Genes in TCGA Samples

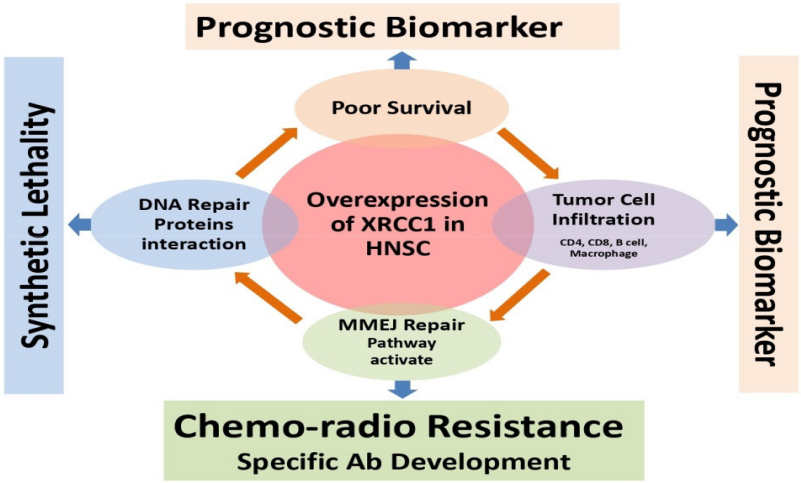


Figure 10. Translational Potential of *XRCC1* as a Prognostic Biomarker

into high- and low-risk groups and performed survival analysis using the KM method (Győrffy et al., 2013). The results showed that the *XRCC1* overexpressing high-risk groups were associated with a poor prognosis (Figure 3), which is consistent with the conclusions of our previous survival analysis.

*Correlation analysis of expression levels of XRCC1and immune cell infiltration*

Correlation with expression level of *XRCC1* and immune cell infiltration in HNSC was analysed using TIMER (cistrome.shinyapps.io/timer). Expression level and infiltration level were significantly positive correlated in our study (Figure 4). Overexpression of *XRCC1* demonstrated that a strong negative correlation with the infiltration level of differentiation CD8+ T cells. Moreover, a CD4+ T cell, B cell, Macrophages, Neutrophils and Dendritic cells shows the significant positive association with the infiltration level (Figure 4).

*Gene-function interaction analysis of XRCC1*

To understand the molecular mechanism of gene expression and their impact on the tumor, we constructed a network of co-expressed genes, including *XRCC1* using STRING. The results showed that shows close association with *XRCC1* given in Figure 5. *LIG3, POLB, NEIL1, TDP1, APTX, PNKP, APEX1, OGG1*, and *XRCC4* genes were significantly associated with *XRCC1*. *XRCC1*shows multiple interactions with *LIG3* and *POLB*, the most significant interactions were physical and pathway interactions.

*Correlation with XRCC1 expression and miR-21*

Expression level of miR-21 was analyzed in TCGA database, as results miR-21 was significantly (P< 0.001) overexpressed in n= 482 tumor tissues of HNSC in TCGA dataset. Moreover, there was no significant (p= 0.26) effect found of miR-21 expression level in HNSC patients survival. That results show the negative correlation because miRNAs regulated the gene expression by suppressing the expression (Figure 6A-B).

*Mutation analysis in XRCC1 and miRNA-21*

Using COSMIC database, we found that there are more than 500 mutations in *XRCC1* coding region that are reported (Figure 7A-B). Amongst these mutations there are three SNPs in mature miR 21 seed region that is responsible for binding the miR21. These mutations in the miR21 binding site are found in HNSC (Figure 7C). Moreover, predictive binding of site of miRNA-21 in human *XRCC1* is on CDC region, and there are huge numbers of mutation present that affect the *XRCC1* regulation by miRNA-21.

## Discussion

Ionizing radiation and alkalytic agents play crucial role in tumor recurrence by affecting the genetic stability. The DNA repair genes are closely associated with tumorigenesis (Borrego-Soto and Ortiz-Lopez, 2015). *XRCC1* is an important member of XRCC family which plays an essential role in DNA repair mechanism (Brem, 2005; London, 2020). *XRCC1* is a key protein of single strand break repair, but recent studies shows it is also an important member of double strand break repair (DSBR) mechanism (Charbonnel et al., 2011; Sharma et al., 2015; Dutta et al., 2017; Eckelmann et al., 2020). *XRCC1* is a scaffold protein assembled the group of protein which will repair the gap, and bind with PARP-1 and 2, *LIG1* and 3, Polyβ etc. *XRCC1-LIG3* complex complete the ligation process (London, 2015; 2020; Tang and Caglayan, 2021). Most of the clinical work related to *XRCC1* gene in cancer has focused on gene polymorphisms in *XRCC1* i.e. Arg399Gln is a genetic susceptibility factor for the HNSC and other cancer (Dutta et al., 2020; Sobiahe et al., 2020; Kabzinski et al., 2021).

In this study using bioinformatics approach we report that *XRCC1* is over expressed in HNSC and this result in poor overall survival. These results are similar with previous studies (Ang et al., 2011; Moreira et al., 2020; Wang et al., 2021) though different from (Yadav et al., 2011). High rate of recurrence and metastases in patients is a major concern affecting morbidity and mortality in HNSC. The reason for the metastasis and recurrence of the disease may be due to the interaction of the surrounding tissues and immune cells that make up the tumor microenvironment (Perri et al., 2020; Wang et al., 2021). We reported that high level of *XRCC1* expression showed a significant negative correlation with the infiltration of the cluster of differentiation CD8+ T cells and positive correlation with the infiltration of the cluster of differentiation CD4+ T cells, B cell, Macrophages, Neutrophils and Dendritic cells (Fan et al., 2021).

It is very useful to understanding the molecular mechanisms of their intrinsic associations with other protein and their collective impact on the tumor. We constructed a network of co-expressed genes and proteins, including *XRCC1* using the STRING publically available database (Szklarczyk et al., 2019; 2021). On visual analysis of protein and gene and 10 genes identified that closely interacted with *XRCC1* (Figure 5). Protein- protein network shows the physical, co-expression, prediction, co-localization, genetic and pathway interaction. *XRCC1* had multiple interactions with *LIG3* and *POLB*; the most significant interactions were physical interactions and pathway. *LIG3* and *POLB* genes are the DNA repair proteins that plays distinct role in DNA repair pathway. For understanding the association of *LIG3* and *POLB* with *XRCC1* we further investigate the expression and survival of these genes, we found that *LIG3* was significantly overexpress in HNSC patient (Figure 8A) and may affect the overall survival (Figure 8C). Although, there was no significant association were found between *POLB* and *XRCC1* expression and survival (Figure 8B, D).

Scaffold protein *XRCC1* play diverse role in base excision repair (BER), SSBR and MMEJ also know as alt-NHEJ. *XRCC1* is consists of three terminal domains including N- terminal domain which is binds to DNA strands break. The C-terminal domain is the central domainconsisting of BRCT-I binds with poly (ADPribose) polymerase 1 (*PARP1*) and BRCT-II binds with to Ligase III (LIG3) (London, 2015; 2020). Moreover, *XRCC1* also interact with other proteins like, *OGG1*, *ATM*, *APE1*, *POLB*, *LIG1, MRE11 and BRCA1* etc., these proteins can be the synthetic lethality partner of *XRCC1* (Table 2). Synthetic lethality therapeutically exploits the inter-gene relationship where the loss of function of either of two related genes is non-lethal, but loses of both causes cell death (Nijman et al., 2011). *PARP1* overexpression in different cancer associated with poor clinical outcome (Rojo et al., 2012; Liu et al., 2016; Li et al., 2018). *XRCC1-PARP1* interaction was well defined in BER and SSBR, *PARP1* has bind on C-terminal domain of *XRCC1* gene. It is noted that high *XRCC1* or high *PARP1* protein level was associated with aggressive phenotype and significantly linked with poor overall survival (Ali et al., 2020). Moreover, pre-clinical gene therapy was selectively toxic in *XRCC1* deficient or platinum sensitive ovarian cancer cells; Ali et al., (2020) concluded that *XRCC1* deficient ovarian cancer cells suitable for synthetic lethality targeting using *PARP1* inhibition. In addition, *XRCC1* deficiency/mutation can also hyper-activate *PARP1* (Hoch et al., 2017).

Double strand breaks (DSBs) repair mechanism is still not fully understood but it can be repaired by different pathways including homologous recombination (HR) and non-homologous end joining (NHEJ) (Scrully et al., 2019). Homologous recombination (HR) is an error-free repair mechanism uses a homologous template for DSBs repair (Sfeir, 2015; Wang et al., 2017) and it is only activated when cells enter S/G2 cell cycle phase because this pathway required cyclin-dependent kinases (CDKs) for promoting end resection for its activation. Although, classical nonhomologous end joining (C-NHEJ)

pathway relies on Ku70/Ku80 and ligates DSB ends without a template and it is activated throughout the cell cycle. Some time DNA double strand breaks repair by another alternative- NHEJ which is called alt-NHEJ or Microhomology Mediated End Joining (MMEJ), this is highly error prone pathway and may associated with aggressive tumor phenotype, recurrence and resistant to therapy. The mechanism of MMEJ activation itself a big research question for scientific community and still under investigation. MMEJ required the proteins which are used in single strand repair mechanism, common are *LIG3*, *POLB*, *PARP1* and *XRCC1*.

Along with *XRCC1*, *FEN1, LIG1* and 3, *PARP1, MRE11*, *NBS1 POLQ*, *POLB*, *RAD50 and Polθ* plays a distinct role in MMEJ repair pathway. Expression of these genes in HNSC cancer patients decides the choice of repair pathways selection. We have analyzed the expression of above mention genes in TCGA RNAseq database; we found that most of them are overexpressed in TCGA HNSC tumor tissues (Figure 9). Furthermore, we analyze the survival of TCGA HNSC patients in reference to MMEJ pathway proteins, we found that *XRCC1*, POLQ and *LIG1* are significantly affected the overall survival of HNSC patients (Table 3). In addition, *XRCC1*could be a biomarker for prognosis and prediction of chemo-radio resistance in head neck cancer patient (Figure 10).

In conclusion, these results provide evidence for using *XRCC1* expression in tumor as prognostic marker for predicting survival in HNSC patients. Understanding how *XRCC1* leads to treatment resistance and modulate immune response can lead to development of targeted therapy.

## Author Contribution Statement

SSA: Drafting the manuscript, data search and analysis, and conceptualizing the MS; SL: Data search and analysis on miR-21; MG: Providing critical inputs for clinical utility of the findings, drafting the manuscript; RC: Providing critical inputs on the search strategy and bioinformatics analysis, drafting the manuscript; AK: Conceptualizing the MS, training SSA and SL for data search and analysis validation of results. Drafting and reviewing the manuscript.

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*Ethical issue*

This study is a part of ICMR-RA ship, and approved by the Institutional Ethical Committee (IHEC) AIIMS Bhopal, India (Ref. No. IHEC-LOP/2021/EF0224).

*Data availability*

*Appendix*

The data used in this study is drawn from the publically available domain and is thus permitted for this study. ULCAN-TCGA (The University of ALabama at Birmingham CANcer data analysis Portal- The Cancer Genome Atlas): This user friendly tool provides access to TCGA, MET500, CPTAC and CBTTC cancer OMICS database and is a good platform for identifying for novel biomarkers, to perform in silico validation of potential genes and provides information on miRNAs expression and patients survival.

Date of use: 30 June 2022

URL: https://ualcan.path.uab.edu/analysis.html

K-M plotter (Kaplan-Meier Plotter): The Kaplan Meier plotter can investigate the relationship between all gene expression (mRNA, miRNA, protein) and survival in over thirty thousand samples from 21 different tumour types, including HNSC, lung, breast, stomach, and colon cancer, and myeloma. The major goal of the tool is to generate and assess survival biomarkers.

Date of use: 2 July 2022

URL: https://kmplot.com/analysis/

SurvivalMeth: A comprehensive web-based automated service for investigating the impact of DNA methylation-related functional elements (DMFEs) on prognosis in various cancer types. It incorporates a variety of combinations, such as single DMFEs, multiple DMFEs, and clinical data, to perform detailed survival analysis and visualization on preupload data, and customised DNA methylation profiles of DMFEs from various diseases to be analysed.

Date of use: 2 July 2022

URL: http://bio-bigdata.hrbmu.edu.cn/survivalmeth/

TIMER (Tumor Immune Estimate Resource): It is a novel statistical web based resource designed for the systematic evaluation of the clinical impact of t immune cells namely B cell, CD4+ T cell, CD8+ T cell, neutrophil, macrophage and dendritic cell that are abundant in tumor microenvironment in different cancers. TIMER provides survival analysis of given immune cells in desired cancer type and correlation analysis of immune cells with the expression of selected gene.

Date of use: 5 July 2022

URL: http://timer.cistrome.org/

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins): This is an online utility tool analyses interaction between proteins and their functional associations along with visualization of partial protein interaction networks and executes gene set enrichment analysis for the entire input.

Date of use: 5 July 2022

URL: https://string-db.org/

miRWalk: This resource offers validated and anticipatory details regarding miRNA-binding locations spanning the 3'UTR, 5'UTR, and CDC region a specified gene of interest across various species, including humans. The method employed for forecasting miRNA target sites relies on a random forest-based technique, specifically implemented through the TarPmiR software. This tool

thoroughly scans the entire gene sequence.

Date of use: 30 June 2022

URL: http://mirwalk.umm.uni-heidelberg.de/

COSMIC (Catalogue of Somatic Mutation in Cancer): This is a comprehensive manually curated and descriptive database that provides detailed information of effects of somatic mutations including non-coding mutations, gene fusions, alterations in gene copy numbers, and mutations responsible for drug resistance in human cancer.

Date of use: 10 Oct 2022

URL: https://cancer.sanger.ac.uk/cosmic

miRNASNP-V3: The miRNASNP-v3 investigates relationships between miRNA-associated SNPs and diseases; analyses impact of SNPs on miRNA-target interactions, expression and their structure; in different cancers, performs functional enrichment analysis to discern miRNA target gain/loss due to SNPs; scrutinizes the links between drug sensitivity and miRNA expression.

Date of use: 12 Oct 2022

URL: http://bioinfo.life.hust.edu.cn/miRNASNP/#!/

*Declaration of competing interest*

The authors declare that they have no competing interests.

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