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Head and Neck Squamous Cell Carcinoma: Prognosis using molecular approach

Review Article

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Abstract: Head and neck squamous cell carcinoma (HNSCC) is the fifth most prevalent cancer worldwide. Apart from various known clinicopathogical factors, it is still a major concern as many genetic and epigenetic alterations bring about the possibility of this deadly disease. The aim of this review is to explore the possible role of DNA repair pathways and the polymorphic status of DNA repair genes (*XPA, XPC, XPD, XRCC1* and *XRCC3*) in the onset of HNSCC, along with sequence variations in genes such as Glutathione S-transferases (*GSTT1*, *M1* and *P1*) that are significantly associated with HNSCC risk. We also focus on the p53 gene mutation induced by various etiological agents and threat factors with its implications towards HNSCC, and emphasise the current therapeutic interventions in treating HNSCC.

Keywords: *Head and neck squamous cell carcinoma • DNA repair genes • Glutathione S-transferases • p53 mutations • Risk factors*

1. Introduction

Head and neck carcinoma or, more preferably, head and neck squamous cell carcinoma (HNSCC) is a heterogeneous class of malignancy originating in the flat, squamous cells that make up the thin surface layer (called the epithelium) in the head and neck region, including the oral cavity, pharynx and larynx [1,2]. HNSCC has been strongly associated with chewing tobacco and slaked lime, betel nut or betel quid chewing, tobacco smoking and alcohol consumption [3]. Although epidemiologically strongly associated with such factors, poor oral health, exposure to human papillomavirus (HPV), exposure to environmental carcinogens and genetic polymorphisms in carcinogen metabolizing enzymes, like glutathione-S-transferases (GSTs) and DNA repair genes have also been found to play a definite role in the prognosis of HNSCC [4].

With the advent of human civilization, there has been an increase in exposure to a large number of environmental chemicals or carcinogens in the form of tobacco, automobile exhaust, pesticides *etc.* Biotransformation results in the activation of various reactive oxygen species (ROS) and reactive nitrogen species (RNS), which then interact with macromolecules. A wide variety of DNA repair systems and xenobiotic metabolizing enzymes (XMEs) have arisen through the evolutionary process to maintain genomic integrity. Ironically, defects in these pathways have been implicated in causing excessive cell death or transformation of cells, resulting in increased risk of HNSCC [5,6]. Polymorphisms in DNA repair genes such as *XPA* (*Xeroderma Pigmentosum Complementation group A*) [7,8], *XPC* (*Xeroderma Pigmentosum Complementation group C*) [9,10], XPD (*Xeroderma Pigmentosum Complementation group D*) [11,12], XRCC1 (X-Ray Cross Complementing group 1) [13,14], XRCC3 (X-Ray Cross Complementing group 3) [15,16] and Phase II xenobiotic metabolizing genes such as Glutathione S-transferases (GSTs: M1, T1 and P1) have been reported in the pathophysiology of HNSCC [1,2,17]. Moreover, any catastrophic change in the p53 gene, along with poor diet and other occupational hazards, can bring about the occurrence of HNSCC

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[18]. This review draws attention to the different polymorphic DNA repair genes, polymorphisms in *GSTs*, and assesses the effects of tobacco, alcohol and other etiologic agents causing mutation in TP53 gene, which ultimately contribute to the risk of developing HNSCC.

2. Genetic polymorphisms with impact on DNA damage and repair

Every day, each human cell is exposed to spontaneous oxidative damages to approximately 10000 bases. However, the failure of the cell's response to DNA damage can lead to cell death or mutations and malignant transformation causing cancer. Evolutionarily, cells are equipped with a number of efficient DNA repair systems to maintain genomic integrity [19]. These systems can be divided into different pathways according to the type of DNA abrasion. At the cellular and tissue level, these biological responses become modified by exogenous and endogenous compounds due to the occurrence of polymorphic alleles in DNA repair genes that alter the repair capacity, thereby developing different kinds of diseases, such as cancer [17]. The polymorphic DNA repair genes involved in HNSCC are grouped into four major DNA repair pathways: Base excision repair (BER), nucleotide excision repair (NER), double-strand DNA breaks repair (DSBR) and mismatch repair (MMR) [12-14].

2.1 Base excision repair (BER)

BER is a major cellular repair pathway that repairs non-bulky lesions resulting from alkylation, oxidation or deamination of bases generating mutagenic and cytotoxic effects, thus correcting single strand intrusions [20]. DNA glycosylase enzymes that are present in large amounts in cells are the main players in recognizing particular DNA abrasions and removing the damaged base out of the DNA helix to fit into the internal cavity of the protein [21]. This creates an apurinic/apyrimidinic (also called AP or abasic) site in the DNA, which can also occur by spontaneous hydrolysis. The mainstay of the BER reaction is the opening of the DNA strand at the abasic site by the APE1 endonuclease. The BER step is also initiated from any single strand break resulting from exposure to radiation or chemicals. In such cases, Poly(ADP-ribose) polymerase (PARP), which binds to and is activated by DNA strand breaks, and the mediator polynucleotide kinase (PNK) are important for protecting and trimming the ends for repair [21]. There are two types of DNA glycosylases: Type I (monofunctional) and Type II (bifunctional). The monofunctional DNA glycosylases (Type I) have no associated apurinic/apyrimidinic (AP) lyase activity and when such enzymes initiate repair, the phosphodiester bond at the abasic site is subsequently incised by the action of AP endonuclease (APE1) interacting with and stimulated by the X-ray repair cross complementing protein 1(XRCC1), which results in a 5'-deoxyribose-5-phosphate (5'dRP) and a 3'-OH. The bifunctional DNA glycosylases (Type II) have an associated AP lyase activity and remove the abasic residue by an endogenous 3' endonuclease activity to result in a single strand break [20]. The BER pathway then proceeds as either short patch BER, characterised by the insertion of a single base at the lesion site, or long patch BER, which involves the re-synthesis of an oligonucleotide consisting of two to ten nucleotides. The short patch BER pathway is dominant in mammalian cells, and when the 5' incision is made by APE1, the abasic sugar is removed by the deoxyribophosphodiesterase (dRPase) lyase activity of DNA Polβ, which further catalyses release of the 5'dRP residues from the incised AP sites by β-elimination. The single nucleotide gap of the DNA backbone is then filled using the action of DNA Ligase III, whose interaction is directed by a noble gene, *XRCCI*, and rectifies the abrasion in the template [20,21]. The long patch BER may be required in the presence of modified AP sites where the 5' moiety cannot be removed by a dRPase activity due to resistance to β elimination. After subsequent strand displacement polβ is dissociated from the damaged site and polδ and polε initiate the repair DNA synthesis, which are in complex with PCNA (Proliferating Cell Nuclear Antigen) and are loaded by RFC (Replication Factor C). Then the FEN1 enzyme (stimulated by PCNA) that carries a 5'-dRP flap endonuclease and a 5'-3' exonuclease removes the displaced DNA flap including the damaged base and ligation of the gap is completed by LigaseI [21,22] (Figure 1).

The *XRCC1* gene (*X-ray repair cross complementing group 1*) involved in the base excision repair pathway has been extensively studied in association with various human cancers [14]. *XRCC1* is located on chromosome 19q13.2, which encodes a 633 amino acid protein and interacts with many components of the base excision DNA repair (BER) pathway, such as PARP-1(Poly-ADP ribose polymerase), PNK (Poly nucleotide kinase), Polβ (DNA polymerase β), LIG3 (Ligase 3α) *etc.* [5], thereby playing an important role in the maintenance of genome integrity. There are three conserved *XRCC1* gene polymorphisms that result in amino acid substitution, detected at *XRCC1 exon 10 codon* ($Arg^{399}Gln$, G>A), *XRCC1 exon 6 codon* ($Arg^{194}Trp$, C>T) and *XRCC1 exon 9 codon* ($Arg^{280}His$, G>A) [23,24]. Reports of *XRCC1* polymorphism associated with HNSCC have been published across the globe. Yuan *et al.* [25]

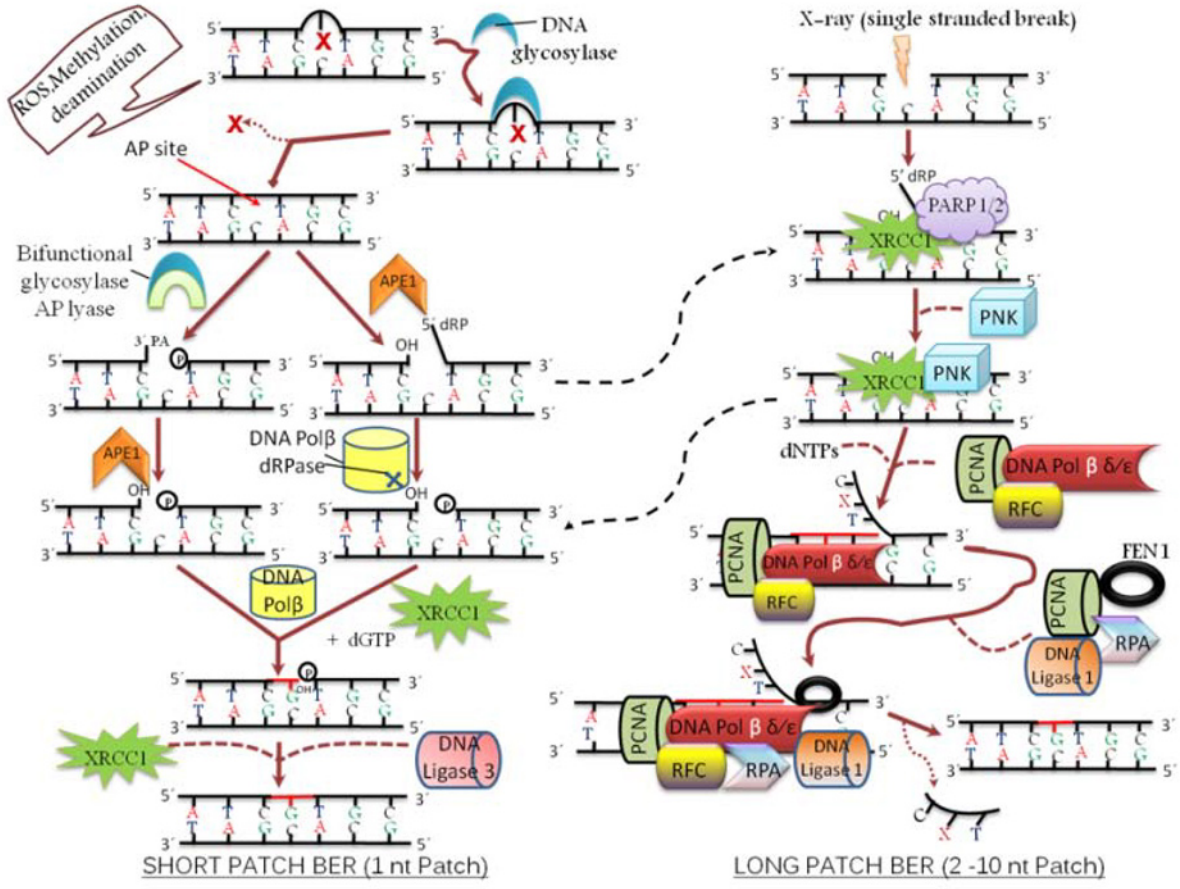

Figure 1. BER pathway. DNA glycosylase removed damaged base. Type I DNA glycosylases lack AP lyase activity, APE1 cleaves the abasic site forming 5'dRP and a 3'-OH. Type II glycosylases have AP lyase activity that removes the abasic residue resulting in a single strand break. PARP and PNK protect SSB and trim the ends for repair. BER follows short-patch/long-patch pathway. Short-patch, dRPase activity of DNA Polβ removes AP site generating a gap, which is ligated by the XRCC1/Ligase III complex. Long-patch, DNA polβ, polδ/polε and PCNA complex repair DNA. FEN1 removes the displaced DNA flap, ligated by Ligase I

conducted a case-control study for a total of 397 HNSCC patients and 900 cancer free controls among the Chinese population and reported that *XRCC1 exon 10 codon* (Arg^{399}Gln) was not significantly associated with HNSCC risk (OR=0.93, 95% CI=0.76-1.13) [25]. Furthermore, there is no significant risk of HNSCC for *XRCC1* Arg^{399}Gln genotypes among non-Hispanic white populations in the USA [26], the Hungarian population [27], or Polish population [28]. Recently, a meta analysis done by Flores-Obando *et al.* [25] observed a statistically significant HNSCC risk for *XRCC1* codon 399 among Caucasians only and *XRCC1* codon 194 variants in the population of New York, USA. A case control study in the Turkish population, with 95 HNSCC patients and 98 healthy controls, reported no significant difference in the frequency of the *XRCC1 exon 10 codon* (Arg^{399}Gln) in the patients and the control groups. However, significant association was observed for the *XRCC1 exon 6 codon*(Arg^{194}Trp) and the risk of HNSCC among smokers [30]. In a Thai population, Kietthubthew *et al.* [31] observed a marginally significant risk in variants with *XRCC1* 194Trp (OR = 1.81, 95% CI = 0.91-3.63, p = 0.09) [31]. More recently, a study on a Pakistani population, including 300 cases and 150 healthy controls, by Mahjabeen *et al.* [32] reported a significant risk of HNSCC for the *XRCC1* Arg^{399}Gln polymorphism. They also reported Tyr^{576}Asn polymorphism to be significantly associated with HNSCC [32]. A study conducted by Kumar *et al.* [33] on a North Indian population observed a reduced risk of HNSCC for *XRCC1 exon 10 codon* (Arg^{399}Gln) and *XRCC1 exon 6 codon* (Arg^{194}Trp) variants in 278 patients and same number of control groups. However, *XRCC1 exon 9codon* (Arg^{280}His) showed no association with HNSCC risk [33]. Khlifi R *et al.*, (2013) reported that only the *XRCC1* Arg^{399}Gln polymorphism was associated with the risk of HNC in the Tunisian population (OR = 2.04; P = 0.001) [34]. Similarly, Mazumder *et al.* [35] reported

smokers carrying the *XRCC1* Arg^{399}Gln genotype to be highly represented among HNSCC patients in Eastern Indian populations [35]. Despite the large number of case control studies conducted on various ethnic populations regarding *XRCC1* polymorphism and the risk of HNSCC, the range of results are still baffling.

2.2 Nucleotide excision repair (NER)

NER is a multi-step process that repairs Bulky DNA adducts, such as UV-light-induced photolesions [(6-4) photoproducts (6-4PPs) and cyclobutane pyrimidine dimers (CPDs)], intrastrand cross-links, large chemical adducts generated from exposure to aflatoxine, benzo[a]pyrene, and other genotoxic agents [36]. There are two branches of the NER mechanism that differ in the transcriptional activity of the gene in the initial recognition step of the repair process. These are: global genome repair (GGR), which repairs DNA lesions over the entire genome, and transcription coupled repair (TCR), which repairs the DNA damage located in the actively transcribed strand of genes by blocking the elongation of RNA polymerase and overall transcriptional activity [21]. During GGR repair, the protein complex XPC-hHR23B (*Xeroderma Pigmentosum Complementation group C* with human homologue of RAD23) and the DNA damage binding protein DDB1 & DDB2 heterodimers first recognise the damaged DNA's helical distortion [22]. During TCR, the DNA lesion is recognised by two specific factors: Cockayne syndrome group B & A (CSB & CSA) displacing the stalled RNA polymerase II for the entry of other repair factors [22]. GGR & TCR then follow a similar process for the rest of the repair pathway. The transcription factor IIH (TFIIH) complex consisting of nine subunits is then recruited to the damaged site with subsequent release of initial recognition factors from the damaged DNA [20]. XPD (5'-3') and XPB (3'-5') which forms the two subunit complex of TFIIH has helicase activity that carries out DNA unwinding throughout the lesion [20]. TFIIH then recruits other subunits, such as XPA and single strand binding protein RPA (replication protein A) to the damaged site. XPA probes for abnormal backbone structure at the damaged site, thus verifying the NER complex, while RPA binds to the undamaged strand and stabilises the unwound DNA [21]. The DNA strand containing the lesion is removed by two endonuclease XPGs that cleave the 3´of the lesion, and excision repair protein 1 (ERCC1)/XPF that cleaves the 5´of the lesion. Resynthesis of DNA occurs by Polδ or Polε and ligation is performed by DNA Ligase I in association with the replication factors PCNA (proliferating cell nuclear antigen) and RFC (Replication factor C) [20,36,37] (Figure 2).

It has been reported that xeroderma pigmentosum (XP) syndrome occurs due to germ-line mutations in the core NER repair genes (*i.e.*, *XPA, XPB, XPC, XPD, XPE, XPF*, and *XPG*) that severely alter their protein functions, and further genetic variation of the NER genes may be used as biomarkers in association with HNSCC [37].

The *Xeroderma Pigmentosum Complementation group A* (*XPA*) gene located at chromosome number 9 (9q23.3) encodes a hydrophobic zinc finger protein present in the nucleus and involved in both the global genome and transcription coupled repair functions of the NER pathway [38]. XPA binds with single stranded DNA binding protein replication protein A (RPA), transcription factor TFIIH, excision repair cross complementing group 1 (ERCC1), xeroderma pigmentosum group F (XPF), and mediates damage recognition [38]. Previously, studies have shown that polymorphism in XPA influences the stability of mRNA transcription factors and may play a crucial rule in susceptibility to cancer [38]. *XPA* single base substitution (G>A) was identified in the 5' non coding region and is the most widely reported polymorphism associated with different cancer [39]. Recently, a hospital based case control study for a total of 154 cases and 105 controls conducted by Bau *et al.* [40] on a Taiwanese population observed that there was no significant risk of HNSCC associated with the *XPA* A^{23}G polymorphism, with oral cancer having the weakest association [40]. Abassi *et al.* [8] reported a case control study on a Caucasian population for a total of 248 cases and 647 controls. They studied polymorphism in different NER genes, including *XPA*, and found no significant risk in association with HNSCC, with particularly weak association in laryngeal carcinoma [8]. In a Japanese population, for a total of 122 cases and 241 controls, Sugimura *et al.* [41] reported that *XPA* polymorphism is significantly associated with oral squamous cell carcinoma (OSCC) risk [41]. A study conducted on upper aerodigestive tract (UADT) cancers, comprised of those affecting the oral cavity, pharynx, larynx and oesophagus, by Hall *et al.* [7] suggested a protective effect of the *XPA G23A* variant genotype in association with HNSCC [7]. Although several studies around the world have reported on the role of *XPA* polymorphism in HNSCC risk, the results are not satisfactory due to different ethnicity, environment, and other factors, and thus further research needs to be carried out in this respect.

Xeroderma Pigmentosum Complementation group D (*XPD*), also known as *Excision repair cross complementing group 2*(*ERCC2*) encodes an ATP dependent DNA helicase that is involved in the NER pathway and present on the long arm of chromosome

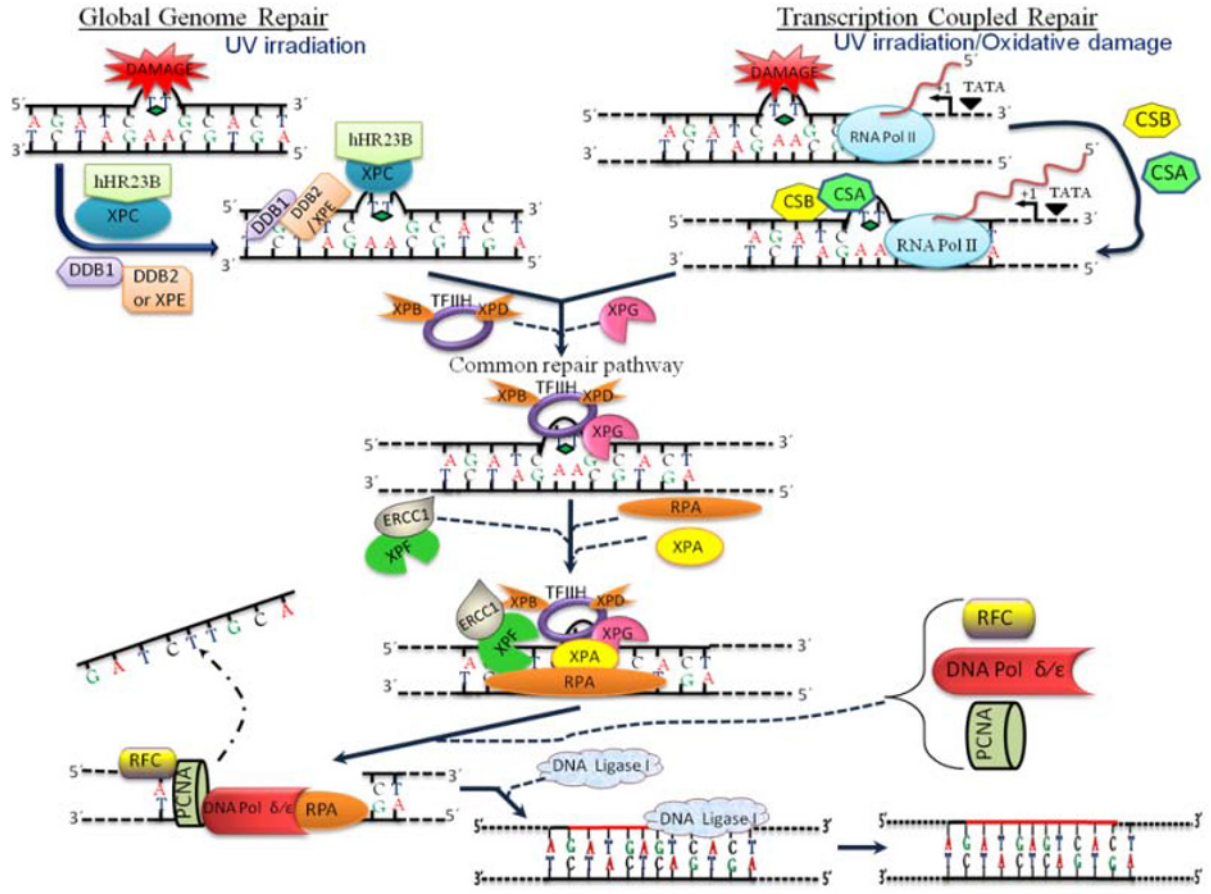

Figure 2. NER pathway. GGR, XPC-hHR23B complex and DDB1 & DDB2 along with XPE recognise DNA damage. TCR, DNA lesion is recognised by CSB and CSA that releases RNA polymerase II. Subsequent stages of both GGR & TCR are identical. TFIIH and its subunits XPD and XPB open 30bp of DNA around the damage. RPA stabilises the unwound DNA and two endonuclease XPG and ERCC1/XPF remove the lesion-containing DNA strand on the 5' side. The DNA synthesis mechanism then completes the repair by DNA Polδ/ε in association with replication factors PCNA and RFC and finally ligation is completed by DNA Ligase I

(19q13.3) [42]. Belonging to the RAD3/XPD subfamily of helicases, the XPD protein is an integral member of the basal transcription factor TFIIH complex that operates in transcription-coupled NER with its ATP dependent helicase activity [43]. Mutations in the *XPD* gene result in three different disorders: xeroderma pigmentosum, trichothiodystrophy, and Cockayne syndrome [43]. Recently, several studies have suggested that *XPD* gene polymorphism is linked with various types of cancer, including those of the head and neck. A population based study in Austria performed by Gugatschka *et al.* [29] reported on the genetic variants *XPD* Lys^{751}Gln (rs13181), *XPD* Asp^{312}Asn (rs1799793) and *XRCC3* Thr^{241}Met (rs861539) for a total of 169 patients (HNSCC) and 463 healthy control subjects. They observed that carrying the *XPD* (Lys^{751}Gln), Gln/Gln genotype was associated with HNSCC (OR=0.54; 95% CI = 0.35–0.92; P = 0.006) [44]. In another case-control study conducted by Harth *et al.* [13] on 312 cases and 300 controls, the *XPD* (Lys^{751}Gln) C/C genotype and combined *XPD* (Arg^{156}Arg) C/A and A/A genotypes were predominant in HNSCC patients [13]. In a non-Hispanic white population, Sturgis *et al.* [11] reported *XPD* (Lys^{751}Gln, rs13181) gene polymorphism to have significant positive association with HNSCC risk [11]. Such results have also been reported by Ramachandran *et al.* [5] and Sliwinski *et al.* [44] on South Indian and Polish populations, respectively [5,44]. Huang *et al.* [15] studied the risk of head and neck cancer in relation to common non-synonymous single nucleotide polymorphisms in various DNA repair genes including *XPD* (Lys^{751}Gln) among 555 cases and 792 controls in western Washington State, North Carolina, and Puerto Rico. They observed that *XPD* (Lys^{751}Gln) gene polymorphism was not significantly associated with HNSCC risk [15]. In another study on a Thai population, Kietthubthew *et al.* [31] reported *XPD* (exon 6 Lys^{751}Gln) polymorphism to have marginal significant risk (OR = 1.71, 95% CI = 0.93-3.16, p = 0.09) of oral squamous cell carcinoma [31]. However, a case control study

for a total of 278 HNSCC patients and the same number of control groups in a North Indian population by Kumar *et al.* [33] suggested that *XPD* ($Arg^{751}Gln$) polymorphism is associated with increased risk of HNSCC [33].

Xeroderma Pigmentosum Complementation group C (*XPC*) is localised in the short arm of chromosome 3 (3p25) and plays a major role in the repair of potential carcinogenic abrasion by participating in the global genome repair of the nucleotide excision pathway for initial recognition of DNA damage [45]. XPC-hHR23B complexes recognize the initial DNA damage in global genome repair and recruit TFIIH at the site of damage [46]. The most studied polymorphisms in the *XPC* gene include (1) a substitution of alanine for valine in codon 499 ($Ala^{499}Val$) in the interaction domain of XPC with hHRAD23; (2) a substitution of lysine for glutamine in codon 939 ($Lys^{939}Gln$), located in the interaction domain with TFIIH; and (3) a poly AT region on intron 9 [45]. Recently a population based case-control study for a total of 829 HNSCC patients and 854 healthy controls among non-Hispanic white Americans showed the *XPC exon 8* ($Ala^{499}Val$) homozygous variant $Val^{499}Val$ genotype to be strongly associated with significant risk (OR=1.57, 95% CI=1.09-2.27) of HNSCC [37]. In another study published by Francisco *et al.* [45] it was reported that interactions of the *XPC* ($Lys^{939}Gln$) homozygous variant Gln/Gln genotype and tobacco exposure may lead to an increased risk of head and neck cancer [45]. A hospital-based case-control study in a non-Hispanic white population conducted by Shen *et al.* [9] for a total of 287 HNSCC patients and 311 control samples reported a newly identified variant allele of *XPC, XPC-PAT+*, to be associated with HNSCC risk, warranting further research [9]. In a study conducted by Wang *et al.* [46] it was reported that the variant T alleles for *XPC* ($Ala^{499}Val$) polymorphisms were linked to a 0.63-fold decrease in OPL (Oral Premalignant Lesion) risk associated with tobacco exposure and an increased risk of oral cancer [46]. *XPC* and *XPD* gene polymorphisms have been analyzed around the world in various case–control studies in association with cancer risk, but the results are still contradictory and further studies need to be conducted.

2.3 DNA double strand breaks (DSB) repair

Probably the most dangerous damage to genetic material is the DSB, which results from exogenous agents such as ionizing radiation (IR), certain chemotherapeutic drugs, and endogenously generated reactive oxygen species or mechanical stress on the chromosomes [47]. They may also arise endogenously during DNA replication or as initiators of programmed processes, such as meiotic exchange and V(D)J recombination of immunoglobulin genes [48]. In eukaryotic cells, there are two evolutionarily conserved pathways for DNA DSB repair: the **HR** (homologous recombination) pathway, which involves the repair of DNA double strand breaks through homologous sequence alignment, and the **NHEJ** pathway (non-homologous end joining), which repairs broken ends with little or no requirement for sequence homology, and is subsequently more error-prone [48].

In the **HR pathway**, the DNA DSB ends are first resected in the 5'- 3' direction, facilitated by the MRE11-RAD50-NBS1 (MRN) complex that possesses an endonuclease and 3'- 5' exonuclease activity on each side of the break, which is a feature of all homology-directed repair pathways [49]. DNA unwinding is assisted by the RAD50 subunit. The 3' single stranded DNA overhangs that resulted from resection are then coated with RPA. RPA facilitates assembly of a RAD51 nucleoprotein filament that probably includes the RAD51-related proteins XRCC2, XRCC3, RAD51 B, C and D [21]. Another protein, RAD52, then interacts directly with RAD51 in facilitating homology searching and strand invasion. A DNA-dependent ATPase, Rad54 (ATRX), also interacts directly with RAD51 and arouses its activity [47]. By displacing a DNA strand, a so-called D-loop is formed by the process of strand invasion and formation of heteroduplex DNA. Strand invasion is then followed by DNA synthesis beyond the original break site to restore the missing sequence information at the break point [49]. The ends are ligated by DNA ligase I in meiotic cells, and the interwound DNA strands (Holliday junctions) are resolved by resolvases resulting in either crossover or non-crossover gene conversion products [47]. The human tumor suppressor protein BRCA1/BRCA2 interacts with Rad51 and also has a distinctive role in the HR pathway. The Rad51 strand exchange activity is modulated by c-Abl tyrosine kinase through phosphorylation. BRCA1 and c-Abl are phosphorylated by Ataxia Telangiectasia Mutated protein (ATM) [47].

In the **NHEJ pathway**, the first DNA binding protein is the Ku heterodimer complex, composed of two subunits: Ku 70 (XRCC6) and Ku 80 (XRCC5), which bind the broken DNA termini and recruit DNA-dependent protein kinase (PKcs), a serine/ threonine kinase [47]. Apart from the Ku heterodimer and DNA PKcs, other proteins involved in NHEJ in mammalian cells are Artemis, XRCC4 (X-ray complementing Chinese hamster gene 4), DNA ligase IV, XLF (XRCC4-like factor; also called Cernunnos, NHEJ1), DNA polymerases μ and λ, PNK (polynucleotide kinase) and WRN (Werner's Syndrome helicase) [50]. PKcs autophosphorylate themselves as well as several other targets, including the Ku subunits, Artemis, XRCC4, WRN *etc* [22].

Artemis possesses 5'→3' exonuclease activity as well as endonuclease activity towards DNA-containing dsDNA/ssDNA (single-stranded DNA) transitions and DNA hairpins in the presence of DNA-PKcs and ATP. Further, it also removes 3'phosphoglycolate groups from DNA ends *in vitro* [50]. PNK executes a 3'-DNA phosphatase and 5'-DNA kinase activity and interacts with XRCC4. Aprataxin and polynucleotide kinase-like factor (APLF) have both endo- and exonuclease activities while WRN (3'→ 5' exonuclease activities) interacts with DNA-bound Ku and XRCC4 or the XRCC4/ Lig IV complex [50]. Finally DNA Pol μ and Pol λ are recruited to the DNA DSB via the interaction with Ku and the XRCC4/ Lig IV, thereby fixing the DNA damage [50]. The Ligase IV function is enhanced by XLF/Cernunos factor (NHEJ1) [51]. The helicases and exonuclease activities of the Rad50-Mre11-Nbs1 (RAD50-MRE11A-NLRP2) complex may also participate in the NHEJ pathway if the DNA ends need processing before ligation [47] (Figure 3).

X-ray repair cross complementing group 3 (*XRCC3*), residing in the long arm of chromosome 14 (14q32.3) [42], encodes a protein that interacts with the Rad51 subfamily and plays a crucial role in the homologous recombination of the DNA double strand break repair pathway [52]. XRCC3 is a key subunit for DNA binding that involves the XRCC3-RAD51C complex; in mammals, *XRCC3* mRNA is expressed in the brain, testis and spleen [53]. Genetic polymorphism in the XRCC3 gene has been a prime focus in HNSCC studies around the world. The single base substitution of Thr^{241}Met at exon 7 (XRCC3-18067C>T, rs861539) is the most widespread polymorphism in *XRCC3* [52]. Werbrouck *et al.* [16] conducted a case-control study on a Caucasian Belgian population to analyse the relationship between DNA double strand break (DSB) repair gene polymorphism and HNSCC. They included 5 single nucleotide polymorphisms (SNPs) in the HR DNA repair pathway comprised of the *XRCC3* and *RAD51* genes, and 4 SNPs in the NHEJ

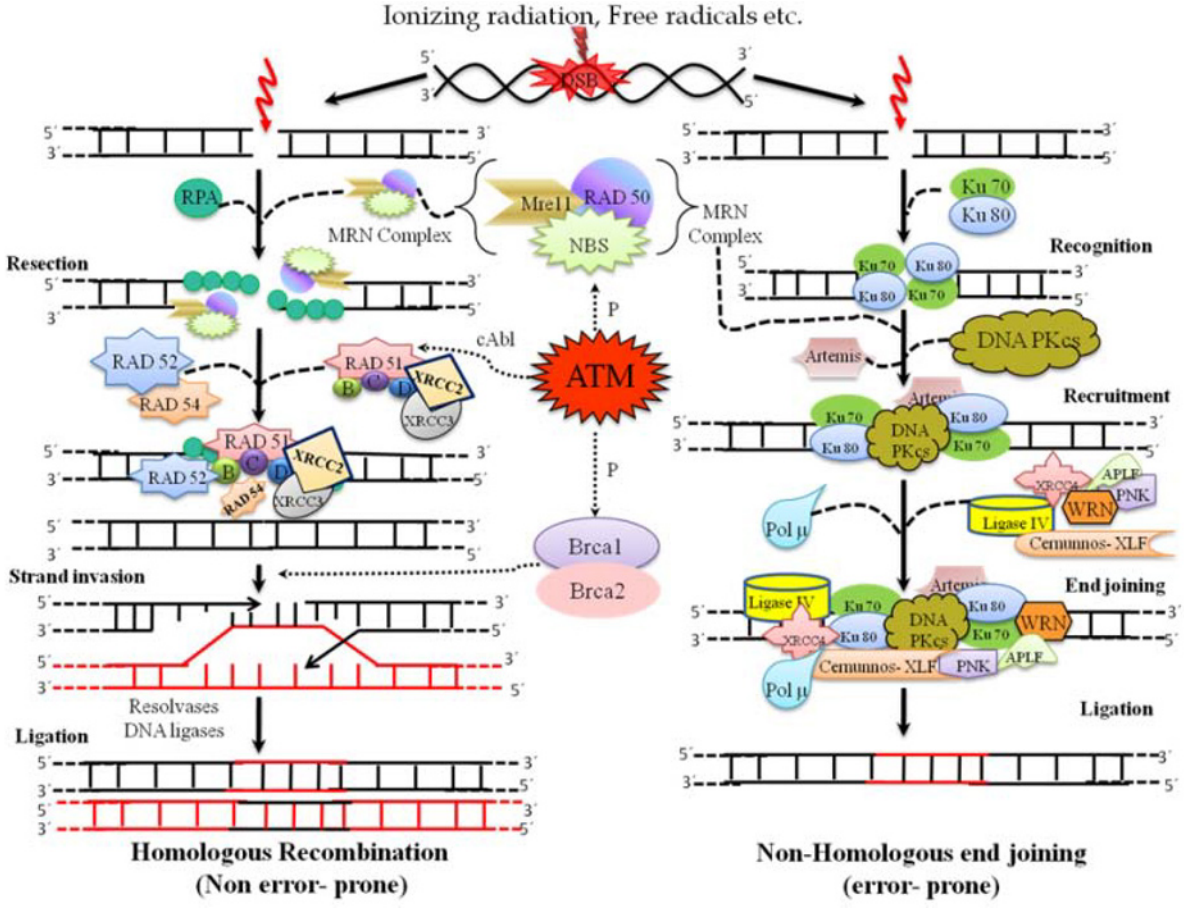

Figure 3. DSB repair pathway. The 5'-3' exonuclease activity of MRN complex creates strand invasion and ATM phosphorylation on each side of the break. RPA assists the assembly of RAD51-B to D, and XRCC2 and XRCC3. RAD52 stimulates filament assembly while RAD54 identifies the homologous sequence. Finally, the resolution of Holliday junctions is completed by resolvases and DNA ligases. NHEJ, Ku and its subunits Ku70(XRCC6) and Ku80(XRCC5) bind the broken DNA termini, recruiting PKcs and Artemis. XRCC4 allows the Ligase IV along with XLF/Cernunos factor, APLF, WRN, PNK, and Polμ to bind DNA and ligate both DSB ends

DNA repair pathway comprised of the *LIG4, Ku70* (*XRCC6*) *and Ku80* (*XRCC5*) genes. They reported the *XRCC3* 722 C>T (Thr^{241}Met, rs861539) variant allele to have a strong significant (odd ratio OR=1.96, p=0.02) association with increased risk of HNSCC [16]. Moreover, a meta analysis done by Yin *et al.* [52] for a total of 3191 cases and 5090 controls in different groups of Asian, Caucasian and other populations, reported an association between *XRCC3*Thr^{241}Met genotypes and HNSCC risk (OR=1.243, 95% CI=1.001-1.544) in all subjects [52]. However, there was no consistent result reported earlier for the XRCC3 722C>T (Thr^{241}Met) variant genotype associated with HNSCC risk in the American population [15,54], which may be due to different ethnicity and environmental factors. In another study performed by Wen *et al.* [15] on a Chinese population for a total of 175 laryngeal or hypopharyngeal carcinoma patients and 525 cancer free controls, the *XRCC3* 241Met variant allele was frequently present in both laryngeal and hypopharyngeal carcinoma cases [55]. Another case-control study in a Thai population, with a total of 106 oral squamous cell carcinoma (OSCC) cases and 164 healthy controls, reported the occurrence of the *XRCC3* 241Met variant allele to be highly significant (OR=2.92, 95% CI=0.94-9.04, p=0.06) with OSCC in association with tobacco and alcohol exposure [31]. Recently, Sliwinski *et al.* [56] conducted a case-control study in a Polish population for a total of 191 HNSCC patients and 97 precancerous hyperplasic laryngeal lesion (PHLL) patients with 353 healthy controls, and reported an association between 722C>T (Thr^{241}Met) *XRCC3* and 3429G>C (G135C) *RAD51* polymorphism and the incidence of HNSCC or PHLL. From their study, it was found that the combination of the 722CT/135GC (OR 3.81; 95% CI 1.55-9.75) as well as the 722TT/135GC genotypes (OR 5.33; 95% CI 1.96-14.47) carried an increased risk of PHLL, with the same gene combinations showing a higher probability of HNSCC occurrence respectively (OR 2.42; 95% CI 1.22-4.79 for 722CT/135GC and OR 3.63; 95% CI 1.69-7.76 for 722TT/135GC) together with tobacco and alcohol exposure [56]. The study conducted by Yang *et al.* [57] provides the first epidemiological evidence supporting a connection between DSB gene variants and oral premalignant lesion (OPL) development. The most notable finding was an intronic polymorphism (A17893G) in *XRCC3*. They compared the polymorphism A17893G with the homozygous wild-type AA genotype; the odds ratios [OR] (95% confidence interval [CI]) for the heterozygous AG and homozygous variant GG genotype were 0.85 (0.49–1.48) and 0.18 (0.07–0.47), respectively (P for trend=0.002). In addition, compared with the most common A-C haplotype of *XRCC3* (in the order of A17893G-T241M), the G-C haplotype was associated with a significantly decreased risk of OPL (OR=0.40, 95% CI 0.23–0.68). Moreover, compared with individuals without the G-C haplotype, the ORs were 1.04 (0.56–1.95) and 0.20 (0.08– 0.51) for subjects with one copy and two copies of the G-C haplotype, respectively (P for trend=0.005). [57]. Despite the large number of case control studies on *XRCC3* polymorphism and HNSCC from different populations worldwide, the results are still not satisfactory, which may be due to various environmental factors, lifestyles, and other variables, and so further large scale population based research needs to be carried out on this subject.

2.4 Mismatch repair (MMR)

MMR was originally identified in bacteria, recognizing and correcting mistakes made by DNA polymerases during replication. This system is mediated by three major proteins: MutS, MutL and MutH, named after their bacterial strains [58]. There is an extensive similarity between the human and the bacterial mismatch repair system, with the major difference being that the human system consists of multiple homologues for each corresponding bacterial component. The homologues *hMLH1* and *hMSH2* are the key components of the human MMR system, recognizing as well as recruiting the additional repair proteins to correct the replication errors that occur during DNA replication [59]. It has been previously reported that the germ line mutation of either *hMLH1* or *hMLH2* is associated with hereditary nonpolyposis colorectal cancer (HNPCC) [58]. However, no study on the association of either of these genes with HNSCC risk has been published [59]. More recently, Jha *et al.* [60] reported that the hMLH1-93 A>G polymorphism is associated with a higher risk of tobacco-related oral squamous cell carcinoma (OSCC) in Asian Indians [60]. However, the results are still baffling and further research needs to be carried out in this regard.

3. Glutathione s-transferases (gstt1, gstm1 and gstp1) polymorphism with emphasis on HNSCC

Glutathione S-transferases (GSTs) are a group of Phase II xenobiotic metabolising enzymes that detoxify various exogenous as well as endogenous reactive species by conjugating mutagenic electrophilic substrates to glutathione (GSH), which can be hydrolysed and easily excreted out from the body [61]. Biotransformation of xenobiotics occurs routinely in our body. The phase I enzymes (including Cytochrome p450 etc) first set up

Nation	Population	Gene studied	Cases (n)	Controls (n)	Polymorphism associated with HNSCC	References
USA	Non-Hispanic whites	*XPC* (Ala^{499}Val, Lys^{939}Gln) *XPD*(Asp^{312}Asn,Lys^{751}Gln) *XPA* (*G23A*) *XPG* (His^{1104}Asp)	829	854	*XPC* (Val^{499}Val)	[37]
Belgium	Caucasian population	*XRCC3* (*XRCC3c.-1843 A>G, XRCC3 c.562-14 A>G, XRCC3 c.722 C>T*)	152	157	*XRCC3 c.722*	[16]
China	Chinese population	*XPD* (Lys^{751}Gln) *XRCC1*(Arg^{399}Gln) *XPG* (His^{1104}Asp)	397	900	Not associated	[25]
Hungary	Hungarian population	*XRCC1*(Arg^{194}Trp, Arg^{399}Gln)	108	-	*XRCC1* Arg^{194}Trp	[26]
Austria	Caucasian	*XRCC1*(Arg^{194}Trp,Gln^{399}Arg, Arg^{280}His) *XPD* (Lys^{751}Gln) *XRCC3* (Thr^{241}Met)	125	463	*XPD* (Gln^{751}Gln)	[29]
Japan	Japanese population	*XPA G23A*	122	241	*XPA G23A*	[41]
India	North Indian population	*XRCC1*(Arg^{194}Trp,Arg^{399}Gln, Arg^{280}His) *XPD* (Lys^{751}Gln)	278	278	*XPD* (Arg^{751}Gln)	[33]
Pakistan	Pakistani population	*XRCC1*(Arg^{399}Gln, Tyr^{576}Asn)	300	150	Arg^{399}Gln, Tyr^{576}Asn	[32]
South Korea	Korean population	*XPC* *XPC-PAT*	73	82	Not associated	[10]
Turkey	Turkish population	*XRCC1*(Arg^{399}Gln, Arg^{194}Trp)	95	98	Not associated	[30]

Table 1. Polymorphisms in DNA repair genes (*XPA, XPC, XPD, XRCC1,* and *XRCC3*) in different populations of the world and risk of HNSCC.

Country	Ethnicity	Gene	Mutation	HNSCC Cases/ Controls	Adjusted OR (95% CI)	P-value	References
USA	American white	GSTM1	GSTM1*0	147/129	0.9(0.5-1.8)	0.278	[66]
Germany	German white	GSTM1	GSTM1*0	312/300	1.0(0.7–1.5)	0.627	[67]
Spain	Spanish white	GSTM1	GSTM1*0	75/200	NS		[127]
Netherland	Dutch	GSTP1	Codon 313 A>G	235/285	NS		[77]
Brazil	Brazilian	GSTM1 GSTT1	GSTM1*0 GSTT1*0	100/100	7.64(1.72-34.04)	0.007	[70]
Italy	Italian Lazio	GSTM1 GSTT1	GSTM1*0 GSTT1*0	100/200	2.61(1.48-4.62) NS	0.001	[68]
Poland	Polish	GSTM1 GSTT1 GSTP1	GSTM1*0 GSTT1*0 105Val	127/151	1.52(0.93-2.49) 2.62(0.64-10.85) 0.97(0.59-1.58)	0.15	[78]
Japan	Japanese	GSTP1	105Val	145/164	NS		[128]
India	North-Indian	GSTM1 GSTT1 GSTP1	GSTM1*0 GSTT1*0 105(Ile/Ile)	175/200	4.47(1.62-12.31)	0.002	[71]
Pakistan	Pakistani	GSTM1 GSTT1	GSTM1*0 GSTT1*0	388/150	2.3(1.5-5.5) 2.04(1.3-3.1)	<0.005	[72]

Table 2. Genotypes of *GSTM1, GSTT1, GSTP1* status and HNSCC risk in different ethnicities.

*NS=Not significant, GSTM1*0 or GSTT1*0=Null genotypes, OR=Odd ratio, CI=Confidence interval.*

functional electrophilic intermediates (such as –OH,-SH, -NH2, *etc.*) in the xenobiotic molecules, which are soon detoxified by phase II enzymes (such as Glutathione S-transferase, GSTs etc) [61]. Human cytosolic glutathione S-transferases (GSTs) are categorized into seven main classes (*Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta*), each including various genes [62]. The most commonly investigated genes of the GST

Country	Population	Studied Polymorphism	HNSCC patients (oral/laryngeal/ pharyngeal)	Wild-type TP53 (%)	Mutant Tp53 (%)	P-value	References
USA	White Hispanic Black	disruptive and nondisruptive mutation	351 20 45	171(48.7) 8(40.0) 16(35.6)	180(58.3) 12(60.0) 29(64.4)	0.630 0.010 0.052	[84]
Brazil	Brazilian	p53exons4-9	90	42(46.6)	48(53.3)	0.527	[82]
India	North Indian	p53exons5-8 p53exons 5-9	53 30	42(79.2) 25(83.3)	11(20.7) 5(16.6)	>0.0001 0.0003	[129,130]
Taiwan	Taiwanese	p53exons5-9	187	96(51.3)	91(48.6)	0.714	[88]
Italy	Italian	p53 intron 3, p53exon4, p53 intron 6	283	191(67.5)	92(32.5)	>0.0001	[87]
Africa	African Black	p53 exons 5-9	55	42(76.3)	13(23.6)	>0.0001	[131]
Thailand	Thai	p53 exons 5-8	68	47 (69.1)	21(30.9)	=0.0016	[132]
England	English	GC-->AT	65	45(69.2)	20(31.0)	=0.0019	[133]

Table 3. Polymorphic status of p53 mutations and HNSCC prevalence in different populations of the world.

superfamily are *GSTT1, GSTM1* and *GSTP1*. Several studies have revealed that polymorphism in these genes may be associated with the risk of HNSCC in different parts of the world [6] (Table 2).

3.1 *GSTM1*

The *GSTM1* gene is located on chromosome 1 (1p13.3), and three alleles have been identified at its gene locus: *GSTM1*A, GSTM1*B,* and *GSTM1*0.* Previously, several epidemiological studies have shown that the null genotypes of the *GSTM1* locus are associated with various types of metabolic disorder, including bladder cancer [63], lung cancer [64] and prostate cancer [65]. In HNSCC, a meta-analysis of 42 published case control studies showed that *GSTM1* null genotypes were associated with a self-effacing risk (OR=1.27, 95% Confidence interval=1.13-1.42) [17]. A case control study on an American white population (frequency 46.3/46.5; OR=0.9, 95% CI=0.5-1.8) reported a similar modest risk associated with *GSTM1* null genotypes among 147 HNSCC patients and 129 controls [66]. Similarly, in a white German population, *GSTM1* null genotypes were found in 53.2% patients with HNSCC (out of 312) and 48.3% of controls (out of 300) [67]. Another case control study in an Italian population by Capoluongo *et al.* [68] reported significant association between HNSCC and *GSTM1* null genotypes in both benign disease controls (p=0.001, OR=2.613; 95% C.I.=1.48-4.62) and healthy donors (p=0.0003, OR=3.35; 95% C.I. 1.69-6.67) [68]. Sato *et al.* [69] found the frequency of *GSTM1* null genotypes significantly higher in oral squamous cell carcinoma (OSCC) cases than in controls in a Japanese population [69]. Leme *et al.* [70] in Brazil conducted a case control study for a total of 100 HNSCC cases and equal number of control groups. They found a significant association between *GSTM1* null genotypes and HNSCC in 66% of patients and 75% of the control group (OR=2.25, 95% CI=1.05-4.84, p=0.0368) [70]. Singh *et al.* [71] found significantly more *GSTM1* null genotypes in HNSCC patients (OR: 2.02; 95% CI: 1.32-3.10; P=0.001) than in control groups in the North Indian study population [71]. Similarly, a case control study in a Pakistani population conducted by Nosheen *et al.* [72] on 388 HNSCC patients and 150 healthy controls reported *GSTM1* null genotypes (OR=2.3, CI=1.5-5.5) had a statistically significant (p<0.05) association with HNSCC risk [72].

3.2 *GSTT1*

The *GSTT1* gene is found on chromosome 22 (22q11.2), and two key alleles occur at the *GSTT1* gene locus: *GSTT1*1*and *GSTT1*0*. Previous studies have revealed that the null genotype or homozygous deletion (0/0) at the *GSTT1* gene locus resulted in loss of enzyme function and may be linked to the risk of HNSCC [73]. A meta analysis done by Hashibe *et al.* [74] reported null genotypes of *GSTT1* have an elevated risk of HNSCC compared with positive genotypes (OR=1.17, 95% CI=0.98-1.40) in different parts of the world [74]. Evans *et al.* [75] reported that the presence of *GSTT1* gene (OR=1.6, 95% CI=1.1-2.5, p=0.03) was associated with a significant increased risk of HNSCC in their US study population. Further, stratified analysis revealed an increased risk for women (OR, 3.0; CI, 1.5-6.3) compared to men (OR, 1.2; CI, 0.7-2.1) for the presence of *GSTT1* [75]. On the other hand, Gronau *et al.* [76] in Germany reported the combined *GSTT1* and *GSTM1*

null genotypes were twice as common in HNSCC patients as in controls (P<0.054) [76].

3.3 *GSTP1*

The *GSTP1* gene is positioned on chromosome 11 (11q13), and has the variant polymorphic genotypes *GSTP1 AB* (*Ile/Val*) and *GSTP1 BB* (*Val/Val*) along with the wild-type *GSTP1 AA* (*Ile/Ile*) [77]. The *GST Pi* polymorphism has a transition of adenine (A) to guanine (G) at nucleotide 313 in exon 5 and results in the substitution of isoleucine (Ile) to valine (Val) at position 104 in the amino acid sequence of the protein [61]. Previously, several studies showed polymorphism of the *GSTP1 105 Val* homozygous allele may elevante the risk of HNSCC, and meta-analysis done by Hashibe *et al.* [74] also revealed that *GSTP1*(*Ile/Val*) or (*Val/Val*) genotypes exhibit enhanced risk (OR=1.10, 95% CI=0.92-1.31) compared to positive genotypes [74]. In a Dutch population, Oude *et al.*, [77] observed homozygous *GSTP1 BB* genotypes in 12.3% patients with HNSCC (out of 235) and 13.6% in healthy controls (out of 285). No statistical differences were found for the *GSTP1 AA*(*Ile/Ile*) and *GSTP1 AB*(*Ile/Val*) or *GSTP1BB* (*Val/Val*) genotypes, which confirmed that *GSTP1* polymorphism is not associated with altered susceptibility to HNSCC [77]. Similarly, Reszka *et al.* [78] found no statistically significant risk of HNSCC in patients carrying *GSTP1 105Val* alleles in a Polish study population (OR=0.97; CI=0.59-1.58) [78].

4. TP53 mutations in head & neck squamous cell carcinoma

Known as the "guardian of the genome", the TP53 protein was first revealed in the year 1979 as a transformation and cellular related protein that assembles in the nuclei of cancer cells and binds strongly to the simian virus 40 (SV402) large T antigen [79]. Later, *TP63* and *TP73* were added to *TP53* gene family [80]. The *TP53* gene is positioned on the short arm of chromosome 17p13.1, which encodes the sequence-specific TP53 protein [79]. TP53 contains 393 amino acids [81] and 11 exons codifying a nuclear phosphoprotein that can specifically bind to DNA sequences, thereby acting as a transcription factor [82]. The TP53 protein, with its unique C and N terminal structure, plays vital role in regulating the cell cycle, cellular proliferation, initiation of programmed cell death, suppressing tumor growth, DNA damage repair pathways, recombination mechanisms, senescence and development [83]. Because of this key role, TP53 has been used as a molecular marker for disease prognosis. However, the role of *TP53* sometimes gets genetically distressed and unable to suppress tumor growth, a loss of function due to genetic mutations—one of the common factors for several cancers, including HNSCC [84]. The majority of human cancers feature a gain of oncogenic function (GOF) or loss of tumour suppressor function (TOF), which are the consequences of missense mutations in the TP53 gene [83]. The thermodynamic stability of TP53 also gets reduced by missense mutations and, as a result, there is a major loss of DNA binding capability and transactivation [85]. The primary single amino acid substitutions reported in different human cancers are Arg175, Gly245, Arg248, Arg249, Arg273, and Arg282 [86], but for HNSCC, Arg72Pro in exon 4 is the only *p53* single nucleotide polymorphism (SNP) whose effect has been studied with contradictory outcomes [87].

Immunohistochemical techniques, direct DNA sequencing, *TP53* gene chips, denaturing high performance liquid chromatography (DHPLC), yeast functional assay [84] and polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) analysis [88] are the different techniques that have been developed to detect *TP53* mutation in HNSCC. Apart from the above mentioned factors, there are others that may lead to *TP53* mutation like tobacco, alcohol consumption, betel quid, occupational exposure, smoke, HPV (Human Papilloma Virus), altered function of Xenobiotic Metabolising Enzyme (XME), radiation, *etc.* [18,89] Among these diverse risk stimulators, tobacco smoking, alcohol consumption [90] and the E6 protein of HPV play key roles in inhibiting the function of the TP53 protein and RB1, which increases the possibility of HNSCC [85]. A number of studies has been already done on different world populations (Table 3) describing the effect of the polymorphic status of *TP53* on HNSCC, and it has been determined that a large number of at-risk populations show their *TP53* gene to be favourably polymorphic with atleast 13 different polymorphic site described by Matakidou *et al.* [91] and Li G *et al.* [92]. In 1996, Paterson *et al.* [93] found that Western populations (47%) are more prone to HNSCC than their Eastern counterparts (7%) by studying TP53 mutation [93]. Other than HNSCC, TP53 mutation results in a higher frequency of lung, bladder, stomach, colon and ovary cancer; furthermore, the deletion of the wild type *TP53* (wt *TP53*) leads to the growth brain, breast, connective tissue, haematological system and adrenal gland tumours [94].

4.1 TP53 pathway

Failure to biotransform xenobiotics leads to adduct formation with DNA, RNA, or cell protein, which can

lead to serious cell damage. Apart from these, DNA damage may result from UV irradiation, alkylation of bases, DNA cross linking, depurination of DNA, alteration of the deoxyribose sugar moiety, reaction with oxidative free radicals and more that may be rectified by multiple DNA damage detection and repair systems in the cell [95]. With the exception of the highly efficient xenobiotic metabolising enzyme system and DNA repair system, every type of damage to DNA is first reported to the TP53 protein and its pathway [95]. DNA damage triggers ATM (ataxia talengiectasia mutated) protein kinase, which activates Chk2 kinase [96] or ATR (ATM and Rad3-related) protein kinase that block DNA replication [97], thereby phosphorylating p53 at distinct sites leading to TP53-dependent cell cycle arrest or apoptosis [98]. Following DNA damage, the TP53 protein elevates endogenous p21 (CDKN1A) mRNA and protein levels, an inhibitor of cyclin dependent kinase (CDK). Over-expression of p21 (CDKN1A) levels blocks cyclin E/CDK-2–mediated phosphorylation of pRb (retinoblastoma protein RBL1) and release of E2F1, a transcription factor, thereby causing cell cycle arrest at the G1-S stage [99]. This gives the cell a chance to repair the damaged DNA during the G_0 phase before entering the S phase of the cell cycle for further proliferation.

In the case of DNA repair failure, TP53 induces a wide variety of genes that participate in TP53-mediated cell death (apoptosis) either by extrinsic (the death receptor) or intrinsic (the mitochondrial) pathways, such as BAX (Bcl-2-associated X protein), FAS/Apo1, IGF-BP3, DR5/Killer (death receptor 5), PIGs (TP53-inducible genes), PAG608 (ZMAT3), PERP (TP53 apoptosis effector related to PMP-22), NOXA (PMAIP1), PIDD (TP53-induced protein with death domain), DRAL, and TP53AIP1 (TP53-regulated apoptosis-inducing protein 1) [100]. TP53 gene mutation hinders the pro-apoptotic BCL2 family member *BAX* gene's expression during apoptosis, resulting in failure of cell death with increased genetic instability and tumour progression [101]. Further, TP53 mutation inhibits the activation of p21 (CDKN1A) causing pRb (RBL1) phosphorylation and subsequent release of E2F1, which inhibits cell

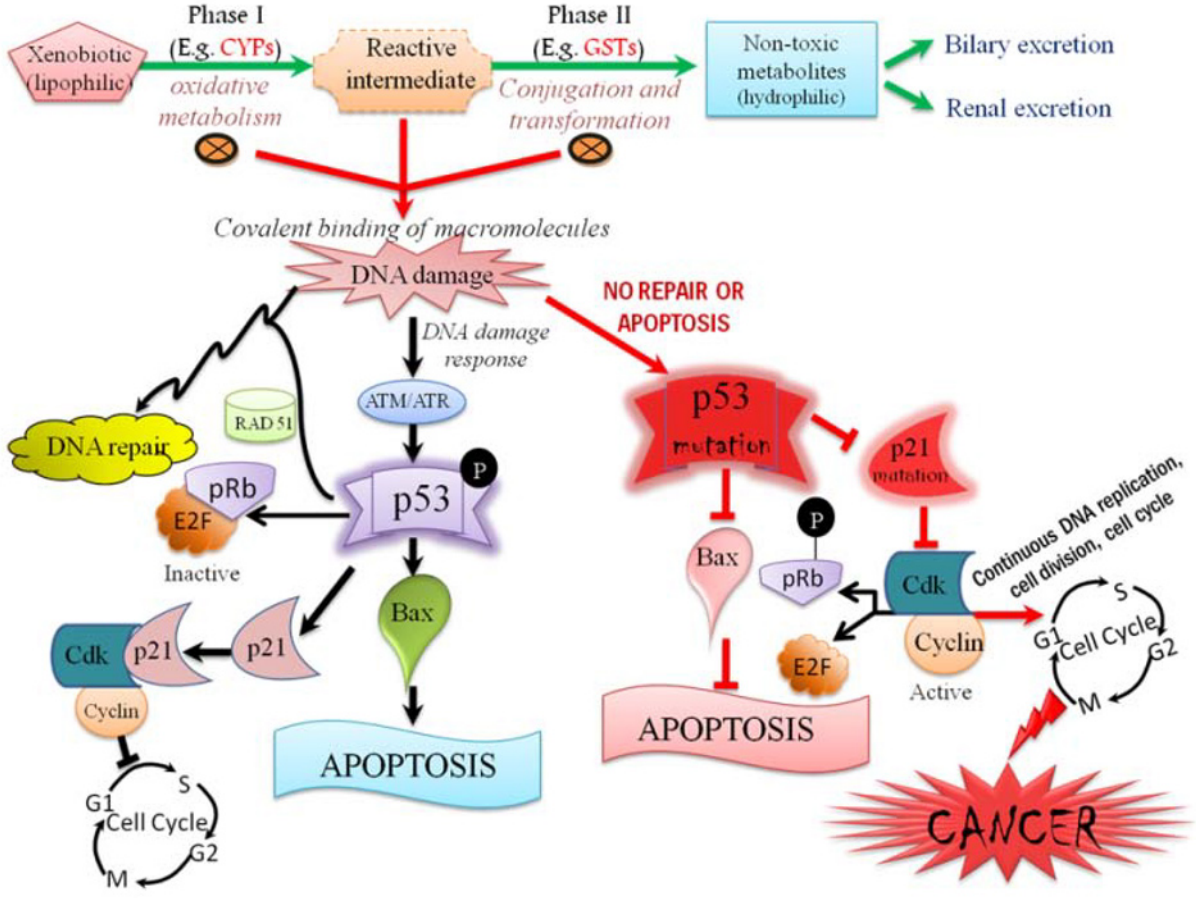

Figure 4. A schematic representation of the p53 pathway and its mechanism of action during DNA damage repair and cell cycle progression.

cycle arrest leading to uncontrolled cell proliferation (Figure 4).

5. Risk factors associated with HNSCC

5.1 Occupational and Diet (Nutrition) as risk factors

5.1.1 Occupational

HNSCC, especially lip cancer, is found to be common among outdoor workers such as farmers and fishermen as they are much more exposed to UV radiation [18]. Another occupational risk factor for HNSCC development is exposure to the by-products of the leather, mining, chemical, agriculture and woodworking industries [102]. Atmospheric pollution by sulphur dioxide and smoke in urban areas has also been shown to raise the risk of developing squamous cell carcinoma (SCC) of the larynx as well as pharynx in parts of England [103].

5.1.2 Diet (nutrition)

Dietary deficiency or imbalance is a risk factor for about 10-15% of oral carcinoma cases in Europe [104]. An epidemiological study in China revealed that the risk of HNSCC, especially oral carcinoma, can be reduced with intake of carotenoids and vitamin C from vegetables and fruits [105]. According to Lucenteforte *et al.* [106] antioxidant vitamins, polyphenols and lignans—which are essential components of fruits, vegetables and whole grains—have been shown to have a negative effect on the development of oral and pharyngeal carcinoma of the head and neck. This effect can be ascribed to the action of antioxidant and carcinogen binding or dilution in the digestive tract [106].

5.2 Tobacco and Alcohol as risk factors

5.2.1 Smoking tobacco

Smoking tobacco in the form of cigarettes, cigars, and cheroots is prevalent in developed as well as in developing countries like India. Cigarette smoke consists of 5000 chemicals, of which 60 compounds are carcinogenic, including nicotine, nitrosamines, polycyclic aromatic hydrocarbons (PAHs; *e.g.*, benzo(a) pyrene), tobacco alkaloids (nornicotine, anatabine, anabasine), and benzene [107].

5.2.2 Smokeless tobacco

Betel nut is generally masticated in either raw or wet form in countries like India. Alkaloids, polyphenols and tannins are the active components of betel nut. Habituated or prolonged use of betel nut may lead to carcinogenic transformation of cells, and there is much evidence to suggest that these effects are associated with increased risk for the development of oral squamous cell carcinoma (OSCC) [108].

Betel quid is generally consumed wrapped in a betel leaf with slaked lime (Calcium oxide/Calcium hydroxide) and other additives like catechu, popularly known as “paan” in India. Earlier epidemiological studies reported that habituated use of betel nut or betel quid with or without tobacco (such as “*gutkha*”) is directly linked with increased HNSCC risk, more particularly oral carcinoma [108]. Zhou *et al.* [109] in New England conducted a population based case control study to evaluate the relationship between smokeless tobacco use and the risk of HNSCC for a total of 1046 cases and 1239 frequency matched controls, and reported that individuals who used smokeless tobacco for more than ten years had high risk of developing HNSCC [109].

5.2.3 Alcohols

In previou *in vitro* studies, it was found that pure ethanol is not carcinogenic; however, when in concert with other environmental carcinogens from tobacco smoking, it leads to an increased risk of HNSCC [18]. Freedman *et al.* [110] conducted a population based study in the USA to investigate any potential relationship between alcohol consumption and risk of HNSCC. They reported a significant dose-dependent risk from alcohol consumption for those who drink more than three alcoholic beverages per day [110].

5.3 Human Papilloma virus (HPV) as a risk factor

Human papilloma virus (HPV), a sexually transmitted infection, is best known for causing cervical cancer, but is also linked to forms of HNSCC, particularly oropharyngeal (tonsils and the base of the tongue), and is detected approximately 25% of all HNSCC cases [111]. In developed countries like the USA, HPV infection is a major cause of oropharyngeal carcinoma. There is evidence that high risk of oral or oropharyngeal carcinoma is associated with sexual behaviour (oral sex) with the presence of high-risk HPV genotypes, such as HPV-16 that plays an essential etiologic role in the development of oropharyngeal squamous cell carcinoma [112].

5.4 Familial and Genetic predisposition as risk factors

Genetic predisposition among family members has been reported for HNSCC in Dutch and Brazilian populations [113]. A possible explanation for the prevalence of cancer within the same family is familial aggregation of

shared risk factors such as genetic polymorphism for carcinogen metabolising genes or detoxifying enzymes [18]. In a study conducted by Yu *et al.* [114] a molecular pedigree analysis of the p16 (CDKN2A) gene locus in blood and tumour DNA from a family was performed. They found a high incidence of HNSCC with a non-functional germline point mutation within exon 2 of the p16 (CDKN2A) gene giving rise to a mutant p16 protein which is formed by substituting proline for the wild type arginine at amino acid position 87 (p16R87P). The mutant (p16R87P) allele segregated with cancer predisposition in tested family members imply a direct causal relationship between the germline p16 mutation in this family with HNSCC tumorigenesis thereby adding p16 (CDKN2A) mutation a new clinical entity for familial HNSCC [114].

6. Current therapeutics on head and neck squamous cell carcinoma

With the commencement of the biomolecular revolution, there has been a tremendous improvement in modern medicine. A recent study conducted by Wolter *et al.* [115] reported that propranolol—a potent beta blocker—along with chemotherapeutic agent cisplatin and γ-radiation has the potential to inhibit HNSCC by initiating apoptosis and repressing the production of vascular endothelial growth factor (VEGF) protein, thereby acting as a novel therapeutic for HNSCC [115]. In recent years the role of polyphenols has come into the spotlight, not only because of their antioxidant potential but also because of their ability to interact with molecular targets within cells [116]. Resveratrol (3,5,4' -trihydroxystilbene), a polyphenolic phytoalexin produced by plants such as grapes, berries, peanuts, and present in red wine has been found to exhibit anticancer properties. Scientific evidence has shown that resveratrol plays an active role in suppressing the proliferation of head and neck cancer including thyroid by increasing cellular profusion of TP53, phosphorylation of TP53 and serine, with upregulation of c-fos (FOS), c-Jun (JUN), and p21Cip1/WAF1 (CDKN1A) mRNAs [117]. Baumeister *et al.* [116] described the antimutagenic properties different polyphenols, especially curcumin (a yellow pigment in turmeric widely used as a spice), as having promising chemopreventive potential for the vast majority of head and neck cancers [116]. Besides having such therapeutic improvement by pharmacological approaches, a sound understanding of HNSCC molecular genetics in accordance with more investigational models, newer strategies for drug therapy, and cost efficacy may play an important role in enhancing positive outcomes.

Of late, surfeits of microRNAs (miRs) in the pathogenesis of HNSCC have been revealed. From the known miRs implicated in various cancer types, MIR-21, miR-107, and miR-34a dysregulation are involved in the progression of HNSCC. miR-107 and miR-34a are downregulated in HNSCC, while MIR-21 levels seem to be upregulated. Ectopic expression of miR-107 and miR-34a and significant downregulation of MIR-21 play important roles in restraining tumor growth, and can be used in HNSCC treatment [118,119]. In the cell line, it has been determined that restraint of either miR-25 or miR-30d increases TP53 expression and promotes apoptosis [120]. However, more research is needed.

It is known that development of tumorigenesis in all cancers entails the activation of oncogenes with subsequent malfunctioning of tumour suppressor genes, such as p53, widely recognised as "the guardian of the human genome" [120]. Restoration of p53 function has been found to cause tumour regression in murine models [121] and can thus be used for better therapeutics against HNSCC in future. Technological advancement over the years has led to the use of monoclonal antibody-based (mAb) therapy for solid tumours that differ from traditional small molecule anticancer drugs and can be used as a new strategy to treat HNSCC [122]. One of the most potent mAbs used for head and neck cancer is cetuximab, whose anticancer properties lie in the blockade of growth factor/receptor interaction and/or downregulation of oncogenic proteins (*e.g.* growth factor receptors) on the tumor cell surface, thereby proving more effective in modulating tumour cells than DNA-damaging chemotherapies and radiation [122].

Pharmacogenetics, a new and exciting entrant in the field of cancer therapeutics, can be used to treat cancer based on each patient's individual genetic makeup. Current oncological practices such as chemotherapy and radiotherapy cause genotoxic side effects. By practising pharmacogenetics instead, individualized therapies and personalised medicine with improved treatment outcomes can be achieved, providing better results in cancer eradication. The current research on oesophageal cancer gives us an ample platform to learn and improve treatment for HNSCC patients in years to come [123].

Radiotherapy is a promising technique used widely for the treatment of cancerous growth but, due to differing genetic makeup among individuals, some patients develop radioresistant tumours. So, to increase specificity, molecular targeted therapy should be administrated along with concomitant radiation in order to increase the response rate (and cure rate) in patients with radioresistant tumours [124].

Recent advancements in identifying the molecular markers that govern the genesis of cancer stem cells (CSC) have provided another novel approach towards cancer therapeutics. CSC departs from current existing therapies and can prove more accountable for treatment failure in several cancers, including HNSCC. To enhance the therapeutic approach, different CSC markers have been developed for HNSCC, like Aldehyde dehydrogenase 1 (ALDH1), CD44 and B-cell-specific Moloney murine leukemia virus insertion site 1 (BMI-1) [125]. However, this strategy still requires further research.

Apart from the above mentioned therapies, a sound knowledge of bioinformatics can also prove beneficial in the diagnosis of HNSCC. The first phase of cancer is often misidentified due to lack of clear symptoms, but with the advent of powerful bioinformatic tools like proteomics, genomics, transcriptomics, metabolomics, peptidomics, glycomics and lipidomics have helped to offset this. The pre-detection of cancer, development of biomarkers, identification of cell growth signals, cell death by apoptosis, and cellular metabolism can be easily established, thereby providing a helping hand [126].

7. Conclusion

Head and neck squamous cell carcinoma (HNSCC) is a serious and frequently lethal disease that affects human populations worldwide. Different population based case control studies have reported that single nucleotide polymorphisms at the *XRCC1*, *XPD, XPA, XPC, XRCC3, GSTM1, GSTT1,* and *GSTP1* loci may be associated with HNSCC, and the occurrence of TP53 mutations also increase susceptibility to cancer. Environmental as well as socioeconomic elements present in a society, like tobacco usage, alcohol consumption, and other etiological factors are strong risk stimulators that may lead to the development of HNSCC. Using knowledge gained from recent research on beta blockers, polyphenols, and other compounds, HNSCC can be prevented to some extent. Particularly, the discovery of miRNAs and their role in upregulation or downregulation of certain genes, with the potential to mitigate HNSCC, is one with profound implications. Increasing familiarity with the recent science of pharmacogenetics adds an additional boost to clinicians in preventing HNSCC. Apart from a sound knowledge of all such measures, HNSCC therapeutics are still in their infancy, and further research along with increased public awareness are needed.

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Conflict on interest statement

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