MAR Dental Sciences

Review Article

P53 Mutation in Tobacco Carcinogen Induced Oral Squamous Cell Carcinoma: A Review

Dr.AnamitraBhowmick*

***Corresponding Author: Dr.AnamitraBhowmick**, BDS, MPH (UK).

Received Date: April 23, 2021

Publication Date: May 01, 2021

Abstract

Tobacco contains more than 60 carcinogens which is the principal risk factor in oral squamous cell carcinoma. Tobacco-associated oral carcinoma morbidity and mortality rates are continuously increasing globally despite of advanced treatment modalities. The poor survival rate in oral cancer patients is attributed to the genetic and epigenetic alterations which are unresponsive to conventional chemotherapeutic and radiation therapy, particularly in advanced and recurrent cases. P53 tumour suppressor is the principal mutated gene in half of the cancers which is the central regulator of 3 billion base pairs human genome playing a significant role in DNA repair and apoptosis of the damaged cells. The molecular basis of p53 mutational patterns in smoking-induced oral carcinoma is poorly understood. This study attempts to screen the existing research evidence base to understand the carcinogenic pathways involved in the interaction of different tobacco carcinogens causing acquired mutation in wild type P53 gene in oral epithelial cells developing oral squamous cell carcinoma. TP53 mutations are detected in all stages of oral carcinoma and it is associated with poor prognosis and reduced survival rate. Due to the mutation in the P53 sequence, it is inactivated and unable to bind with the DNA to inhibit abnormal cell differentiation giving rise to tumorigenesis. A missense mutation in the DNA binding domain of P53 is the most prevalent type of mutation in oral carcinoma.

This study highlighted the key role of the P53 DNA binding domain in oral carcinoma. Tobacco carcinogenswhen chronically exposed to the epithelial cells bind with the segment of DNA forming DNA adducts which triggers P53 mutation in oral mucosa but the molecular mechanism needs further exploration. It was revealed that carcinogens, particularly PAH-induced Guanine to thymine transversions are attributable to P53 mutations in smoking-associated carcinomas. The TP53 mutational patterns in smoke-associated carcinoma are different in comparison to non-smoking carcinoma suggesting the crucial role of carcinogens in pathways involved in TP53 mutation in tobacco-induced oral carcinoma. In recent years P53 has been genetically engineered to be used as gene therapy in recurrent oral cancer patients as an adjunct to conventional treatments because of its tumour suppression mechanism. Multiple clinical trials have been conducted, particularly in China to assess the safety and efficacy of P53 as anti-tumour gene therapy. Based on the findings investigators reported that P53 is safe and effective as an anticancer gene therapy in recurrent patients. Administration of different kinds of genetically engineered P53 transgenes in recurrent oral cancer patients demonstrated tumour regression. This ground breaking invention could pave the way to improved therapeutic target therapy and require further rigorous research.

Introduction

Cancer is the leading cause of death every year even after decades of clinical research and innumerable therapies **(**5**)**. It can be called the king of all life-threatening diseases due to DNA damage-induced genome-wide genetic instability **(**4**)**. Cancer encompasses diverse genetic and epigenetic aberrations and multiple undiscovered regulatory pathways attributing to poor survival rate **(**28**)**. According to the latest estimates of cancer incidence and mortality by GLOBOCAN 2020 produced by the international agency for cancer research; 19.3 million new cancer cases and approximately 10 million cancer deaths were reported across the globe from 185 countries comprising 36 different cancers alone in 2020 **(**58**)**. The continuously rising global cancer burden at an alarming rate is expected to reach 28.4 million cases by 2040. Today cancer is a major public health issue requiring immediate attention to implement appropriate interventions preventing end-stage complications **(**58, 71**)**. Appropriate public health interventions must be implemented globally to increase awareness regarding the harmful effects of smoking and alcohol causing cancer.

Background:

In the last five years, new technological advancements in biomedical research in genomics and bioinformatics like next generation DNA sequencing, CRISPR cas 9 technology, molecular docking, molecular diagnosticsetc. have revolutionized medicine and pharmaceuticals (28). New technologies are unravelling a myriad of unexplored mechanisms in cancer which were difficult to investigate earlier with conventional methods (16). The mechanisms causing invasion, distant metastasis and drug resistance were poorly understood. But, the field of oncogenomics is facilitating the mapping of cancer associated genomic and epigenomic alterations providing a deeper understanding of the evolution and progression of cancer at the sub-atomic level (40). This extraordinary progress in medicine will greatly improve the understanding of the molecular aspect of cancer. Clinical applications of new technology will contribute to the early detection, guided target therapy, and non-invasive monitoring of cancer. Multiple complex mechanisms are yet to be discovered in cancer biology in the coming future for the effective management of cancer (40; 19)

Oral carcinoma: In the new era of cancer genomics

Epidemiologic studies have established a correlation between tobacco consumption and squamous cell carcinoma of the head and neck including oral cancer (20; 9). Oral cancer is one of the most prevalent cancers and a major public health issue (71; 58; 52). According to the data from GLOBOCAN 377,713 new cases (2.0% of total new cancer cases) and 177,757 (1.8% of total cancer deaths) deaths had been reported in 2020. These global cancer statistics are indicating that oral carcinoma is prevalent worldwide and the morbidityand mortality are continuously rising. Most (90%) of the oral cancers constitute Oral Squamous Cell Carcinoma [OSCC] (32). Despite advanced multi-modality therapies including chemotherapy, surgery, intensity-modulated radiotherapy and the newest biological therapies the overall survival rate of oral cancer remains poor worldwide over the years (35). Therefore, novel biomarkers with increased sensitivity and specificity and improved efficiency are required for the early detection of oral cancer (52).

The genomic imbalances caused by the risk factors are poorly understood in oral cancer and precancerous lesions (31, 41). It is imperative to understand the molecular mechanisms of each carcinogen transforming a normal squamous epithelial cell in the oral mucosa into a malignant cell giving rise to aggressive tumorigenesis. The advent of genome-scale analysis technology has facilitated the understanding of the genetic alterations underlying diseases (53). Furthermore, the completion of the Human Genome Project and development of high-throughput omics technology-enabled oral cancer genomic, proteomic, transcriptomic, metabolomic profiling to identify alteration in genes, m-

RNA and proteins characterizing oral squamous cell carcinoma (62). The genomic–wide profiling in OSCC identified deletions of multiple chromosomal regions such as 7p, 8q, 9p, 11q in tobacco-induced oral carcinoma (62). Whilst, in many studies the whole-exome sequencing of cancer cell lines revealed that TP53 is the most commonly mutated gene in the head and neck squamous cell carcinoma (HNSCC) including the oral cavity (35).

Smoking, alcohol and betel-nut: oral cancer enigma

Bad oral habits account for approximately 75% of oral carcinoma (68; 35). It is well established by numerous research studies (38, 33, 60, 37, 51, 55) that deleterious oral habits like persistent tobacco smoking, betel quid chewing and alcohol consumption cause genetic aberrations causing mutations in the genomic sequence responsible for the malignant transformation and progression in oral carcinoma. These three lifestyle factors are the most significant risk factors for oral carcinoma which induce cancer development (32). These risk factors produce acquired genetic mutation due to chronic exposure of the oral mucosal cells to lethal carcinogens. These acquired mutations are preventable by lifestyle modifications. This is unlike the inherited mutation where offspring inherit the mutated copy of the gene from one of the parents (17, 15, 64)

One study conducted a systematic review and meta-analysis of the epidemiological studies which evaluated the impact of smoking on oral cancer to assess the consistency of the tobacco induced oral cancer evidence base and reported increased susceptibility of tobacco smokers to develop oral carcinoma (51). Likewise, another study (48) conducted a meta-analysis of epidemiological studies that investigated the correlation between tobacco consumption and oral cancer and concluded that smokers are more prone to develop oral carcinoma. The most critical gene is p53 which is mutated in approximately 50% of the cancers (49). Researchers consider P53 mutation as an early indicator of developing carcinoma. In contrast, a pre-cancerous lesion from a non-smoker does not exhibit p53 mutation (68). Research evidence analyzed so far is suggesting the association of harmful oral habits with oral carcinoma. Among the three most significant environmental risk factors causing oral carcinoma; tobacco smoking, alcohol consumption and betel nut chewing this article will explore the most significant risk factor tobacco in relation to P53 mutated oral carcinoma.

Aim and objectives:

This review article aims to explore the relationship between the most commonly mutated TP53 gene; carcinogens in tobacco and oral squamous cell carcinoma which accounts for most of the oral cancers.

The objectives of this study are

- To analyze the existing literature to understand the molecular pathways triggered by the carcinogens from tobacco which mutate tumour suppressor gene P53 and its role in transforming a normal epithelial cell into a cancer cell.
- To analyze the difference in the P53 mutational patterns in smokers and non-smokers in oral squamous cell carcinoma.
- To predict future research directions emphasizing P53 mutation in smoking-associated oral squamous cell carcinoma.

Carcinogens in tobacco:

Tobacco is consumed worldwide by millions of people. There are 1.3 billion smokers worldwide consuming tobacco **(**27**)**. This lethal substance comprises principally nicotine and carcinogens making tobacco a significant risk factor accountable for numerous carcinoma including lung, oesophageal, laryngeal, head and neck and oral. Several tobacco carcinogens are N-Nitrosamine, PAHs **(**Polycyclic Aromatic Hydrocarbons**)**, aromatic amines, 1,3-butadiene, benzene, aldehydes, ethylene oxide, etc. **(**24**)**. These tobacco carcinogens cause genetic and epigenetic alteration of oral epithelial cells and their metabolites produces oxidative stress on tissues which induces tumorigenesis **(**27**)**.

Several studies **(**2; 7; 6; 46**)** analyzed pharmacological actions of the major neuroactive compound of tobacco, nicotine. The authors reported that it is an addictive which develops tolerance causing nicotine dependence. This addiction compels people to consume huge amounts of tobacco over years which contain more than 60 carcinogens that induce genetic mutations in tumour suppressor genes by interacting with the cell DNA. Chronic exposure of oral epithelium to carcinogens alters the genomic sequences resulting in dysplasia, anaplasia, carcinoma-in-situ where the behaviour of cells changes and they exhibit poor differentiation **(**24**)**.

Jian et al reported that tobacco carcinogens cause abnormal expression of several genes including P53 resulting in its mutation. Post mutation P53 exhibits loss of all the regulatory effects on DNA repair, apoptosis and cell growth. Another study found that P53 overexpression was higher in the malignant

lesions of smokers in comparison to non-smokers **(**29**)**. A similar finding was reported by other studies also which verified the association of tobacco carcinogens with P53 gene overexpression in oral epithelial cells **(**4; 65; 11; 66**)**.

Furthermore, an observational study reported the overexpressed P53 in patients with a history of smoking and alcohol consumption. It also highlighted that three-fourth of the patients with poorly differentiated oral carcinoma showed p53 overexpression (1**)**. The molecular interaction of P53 with different tobacco carcinogens in the development of oral pre-malignancies and malignancies is poorly understood **(**49.**)**.

P53: The guardian of the genome

P53 is a multi-functional protein exhibiting crucial functions as a central genome regulator including tumour suppression, DNA repair, autophagy, apoptosis, senescence, normal homeostasis and control of oxidative metabolism **(**Jain; 25**)**. The most distinctive property of P53 is its relationship with DNA damage. DNA damage is universally present in all cancers which activate P53. P53 acts as a brake to inhibit the damaged DNA replication forming polymorphic aberrant cells **(**63**)**. That is why P53 is universally known as the "**Guardian of the genome and policeman of the oncogenes**" because of its unique ability to identify the signs of DNA damage; single-stranded or double-stranded breaks triggered through multiple pathways. These pathways include signalling cascade of DNA-dependent protein kinases ATM **(**Ataxia Telangiectasia Mutated**)**/ATR **(**Ataxia telangiectasia Related**)** and Chk1/Chk2 **(**16; 63**)**. Double strand breaks in DNA stimulate ATM which stimulates Chk2 **(**63**)**. These kinases conduct P53 phosphorylation which blocks P53-MDM2 interaction stabilizing P53 **(**63**)**. This pathway is found to be permanently activated in all carcinomas illustrating the intrinsic association between the DNA damage and P53 activation **(**16**)**.

DNA damage activates P53 to initiate the DNA repair mechanism. It induces apoptosis of the damaged cells to prevent abnormal cell proliferation. This mechanism makes it an effective tumour suppressor gene. This tumour suppressor blocks the cell division at the G1 –S phase activating DNA repair mechanism. Oncogenic signalling also activates P53 through another protein ARF **(**Alternative reading frame**)** which in turn activates MDM2 inhibiting the P53-ubiquitin ligase activity which is a negative regulator of the P53 tumour suppressor protein and stabilizes P53 activity **(**16**)**.

P53 is responsible for the upregulation and downregulation of biomolecules impacting carcinogenesis. A proto-oncogene, MDM2, binds directly to P53 repressing its transcriptional activity resulting in P53 proteasomal degradation. Deregulation of P53 instigates genetic mutations and epigenetic

modifications leading to aberrant protein overexpression **(**49**)**. Target protein overexpression causes damaged cells to proliferate abnormally resulting in local invasion and eventually metastasis which is the hallmark of carcinoma. This abnormal proliferation is inhibited by p53 physiologically. This mutation allows damaged cells to divide in an uncontrolled manner in the absence of tumour suppressor P53. Downregulation of the suppressor genes enhances malignant transformation **(**49**)**.

P53 gene is either directly or indirectly inactivated by different mechanisms as a result of genetic alterations. The altered gene transmits information to and from p53 initiating cascades of abnormal signalling pathways **(**63**)**. The inactivating mechanisms could be a viral infection, mislocalization of P53 from the nucleus to cytoplasm, multiplication of the MDM2 gene in the genome, deletion of $P14^{ARF}$ gene and amino acid mutation in the DNA binding domain. Among these mechanisms, an amino acid mutation in the DNA binding domain of P53 gives rise to most cancers like breast, pancreas, urinary bladder, brain, gastrointestinal and many more **(**63**)**. The intricate interaction of P53 with numerous genes at the ultra-microscopic level needs further exploration for the early detection of malignant transformation **(**49**)**. For this, it is quintessential to have an extensive understanding about the molecular domains of P53 which undergo mutation.

P53: Exploring the molecular structure of the tumour suppressor gene

Human p53 encoded by TP53 gene is a 393 amino acids long flexible multi-domain protein with 191 amino acids comprising the central sequence-specific DNA binding domain and 53 amino acids carboxy-terminal tetramerization domain **(**42**)**.

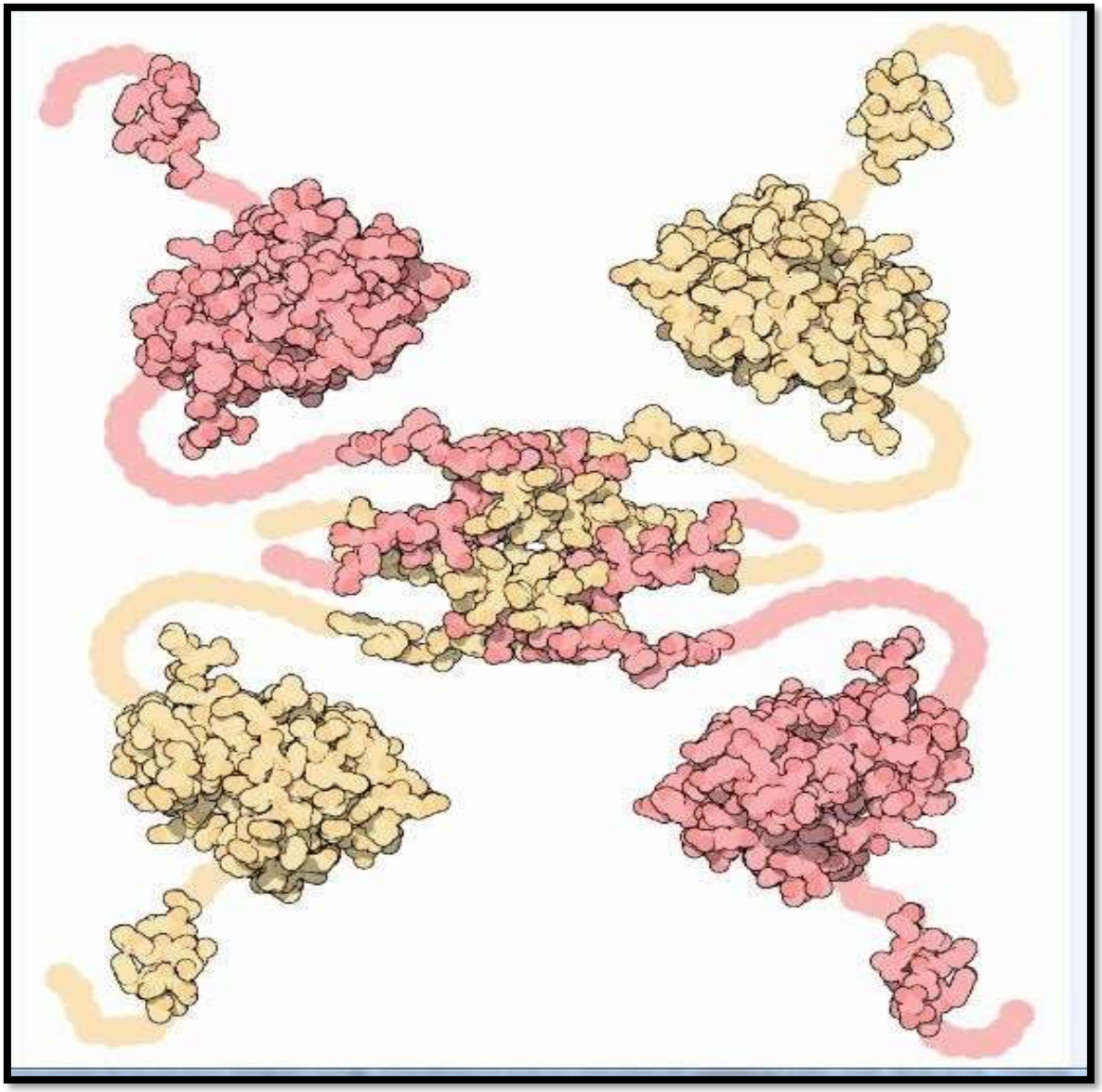

Image source: pdb101.rcsb.org/motm/31

Figure 1: The above picture is illustrating the flexible portions of P53 molecules which bind with DNA and are subjected to mutations. It consists of (a) Central tetramerization domain (b)Four DNA binding domains which is mutation prone (c)Transactivation domain located at the end of every flexible arm.

P53 gene is located on chromosome 17 comprises seven domains for molecular binding. P53 monomers oligomerize to form tetramers which interact with other proteins **(**20.; 42**)**. From a molecular perspective, P53 is a flexible molecule that is composed of 4 identical protein chains **(**Figure.1**)**. The compact globular portions are called "domains". In Figure 1, there is a central part attached to four large globular portions through flexible long chains. The central portion is the tetramerization domain **(**Figure 1, a**)** tied to four long, flexible chains which in turn is connecting to the second domain-DNA Binding Domain [DBD]. These DNA binding domains **(**Figure 1, b**)** demonstrate maximum mutations. Most of the P53 mutations in cancer are found in the DNA binding domain. These domains are rich in arginine residues which interact with the DNA. The most common mutations in DBD are arginine 248 which binds to DNA thus forming a stabilizing P53-DNA complex **(**Figure 2**)**. Mutation at arginine 248 causes loss of stabilization leading to genetic error. Other mutation sites are arginine 175, 249, 273, 282 and glycine residue 245 **(**Figure 2**)**. The most common mutation is the missense mutation which causes an alteration in the DNA at a specific position. This mutation replaces one amino acid with another amino acid changing the genomic sequence. This swapping of the amino acid converts wild-type P53 into a mutant P53 which is unable to inhibit the abnormal cell-cycle. The third domain is the transactivation domain **(**Figure 1, c**)** located at the end of the DNA binding domain at every arm. This domain activates the DNA-reading machinery **(**63**)**.

Pavletich et.al (42) conducted in vitro experiment using proteolytic digestion to locate the sequence-specific DNA binding domain in P53 protein. The experiment demonstrated that the highly conserved middle portion is resistant to proteolysis indicating a compact structural domain. The central core domain constituted a sequence-specific DNA binding domain while the carboxy-terminal contains a tetramerization domain. P53 binds to specific DNA sequences to activate the transcription process **(**42**)**. This transcriptional activity of P53 was located at the amino terminus in residue 1-42 amino acids while the oligomerization activity was detected at the carboxy-terminal portion. **(**42**)**. P53 mutant which has lost its regulatory control on cell-cycle failing to bind DNA start forming hetero-oligomers with wild-type P53 promoting tumorigenesis **(**47**)**

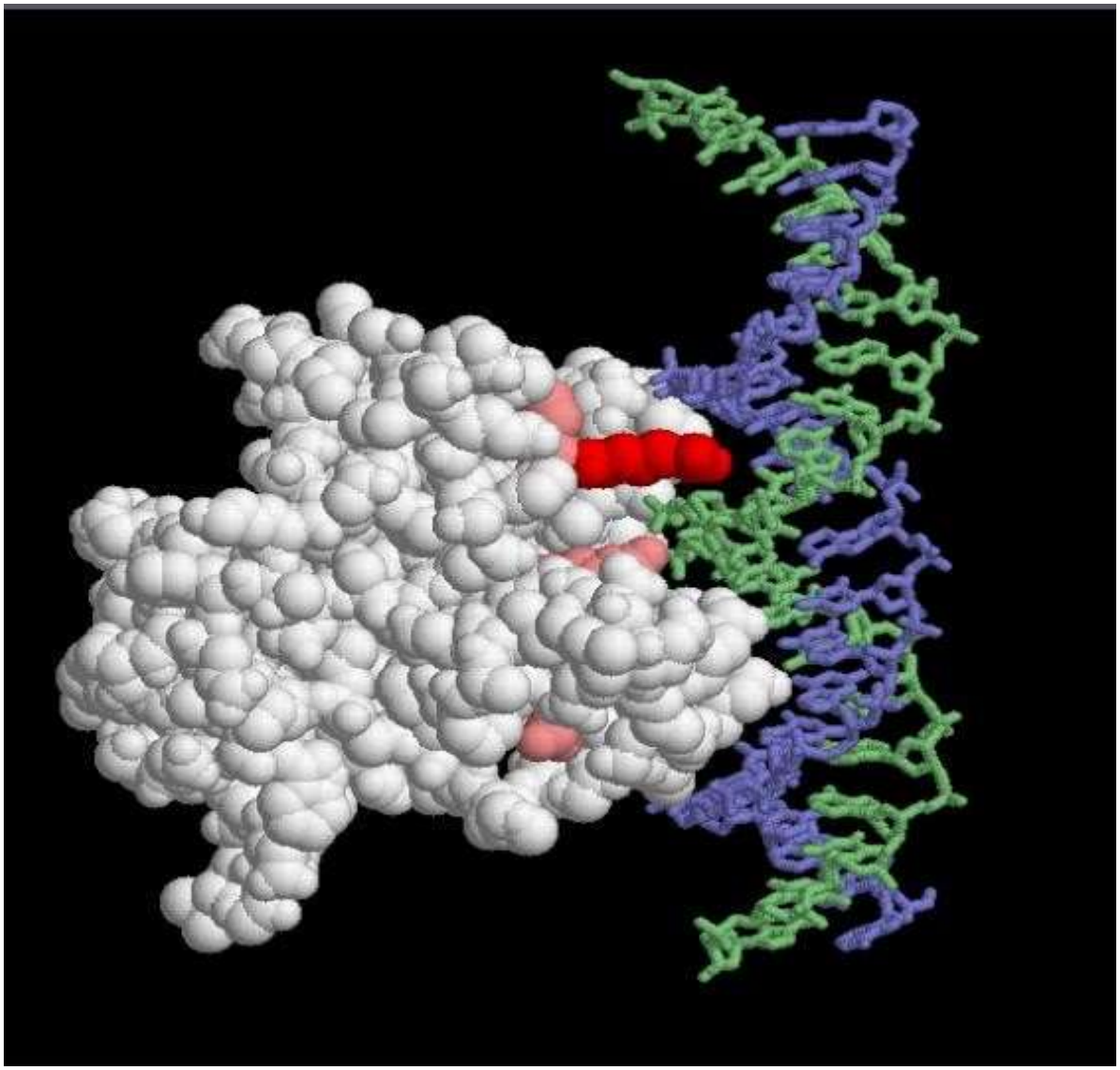

Image source: pdb101.rcsb.org/motm/31

Figure 2: P53 complexed with DNA is illustrating different sites of mutations in the DNA binding domain of the tumour suppressor gene P53 in cancer. The most common mutation is arginine 248 (red-coloured) which binds to the minor groove of the DNA (Blue and green coloured) and other mutation sites are arginine residues 175, 249, 273, 282 and glycine 245 (Pink-coloured)

Very few studies have explored the molecular pathways altering the DNA sequences due to the action of various carcinogens from cigarette smoking leading to P53 mutation in oral cancer. Also, the causes of P53 mutation particularly in tobacco-associated oral carcinoma are less discussed and poorly understood in the existing literature.

P53 mutations in oral carcinoma:

The development of OSCC involves multiple genetic events which deregulate the functions of proto-oncogenes and tumour suppressor genes and DNA repair genes resulting in highly disorganized signalling pathwaysin a normal cell which causes mutations in transcription factors **(**31**)**. Several oncogenes and tumour promoting genes have been implicated in oral cancer including *cyclin family, ras,PRAD-1, RB1, cyclin-dependent kinase inhibitors and* ***P53*** **(**31.35; 29**).** Lindemann et al; Kaur et al demonstrated the tumor suppressing actions of P53 and its significant role in the development and progression of oral squamous cell carcinoma. DNA damage is generated by ionizing radiation and chemotherapy which activates P53 gene to inhibit DNA damage **(**35**)**. However, mutation in P53 leads to a series of genetic mutations in oral cancer. P53 is unable to bind DNA triggering the cell cycle arrest **(**42**)**. It has been found that P53 is expressed in all OSCC **(**31.**)**

P53 mutation modifies specific molecular pathways increasing the risk of cancer development **(**30**)**. Hence, an aberration in chromosome 17 plays a significant role in the pathogenesis of carcinoma **(**49**)**. It is evident from the results of various clinical trials that P53 mutation is present in all kinds of head and neck squamous cell carcinoma including oral cancer. The different kinds of P53 mutations observed in OSCC are missense, nonsense, frameshift, inframe deletions, splice site and stop gain mutations which develop carcinogenetic changes in a normal oral epithelial cell. The author reported that approximately 70% of oral squamous cell carcinoma exhibit P53 mutations. These mutations alter the cellular phenotype causing accelerated and unchecked cell growth resulting in malignant transformation of the oral tissue **(**49**)**.

Therefore, TP53 mutations have been detected in all the stages of Head and neck cancer from development, progression, metastasis to disease prognosis. Mutated TP53 has been detected in poorly differentiated advanced carcinoma cases predicting poor prognosis. Studies suggested a reduced survival period in cancer patients with mutated TP53 gene in comparison to patients with wild-type TP53**(**32**)**. However, the prognostic impact of TP53 mutation on OSCC prognosis requires further exploration.

Lapke et al reviewed a large observational study **(**Poeta et al**)** comprising of 560 patients of Head and Neck Squamous Cell Carcinoma [HNSCC]. This study analyzedthe mutational patterns of TP53 genes among the patients. Major kinds of P53 mutations detected were missense, stop-gain, splice-site; frameshift deletion and inframe deletions **(**Figure 3**)**. The most notable among these is the missense mutation in the DNA binding domain with the highest frequencies recorded in hotspots R273, R248 and R175 (R depicting arginine residues). A study reported about P53Arginine72 pro polymorphism contributing to the pathogenesis of oral cancer **(**49**)**. These mutations revealed a stop codon in the specific DNA binding domain. These are non-conservative mutations called disruptive TP53 mutations causing loss of activity and are associated with invasion, poor prognosis and decreased survival rates **(**32**)**.

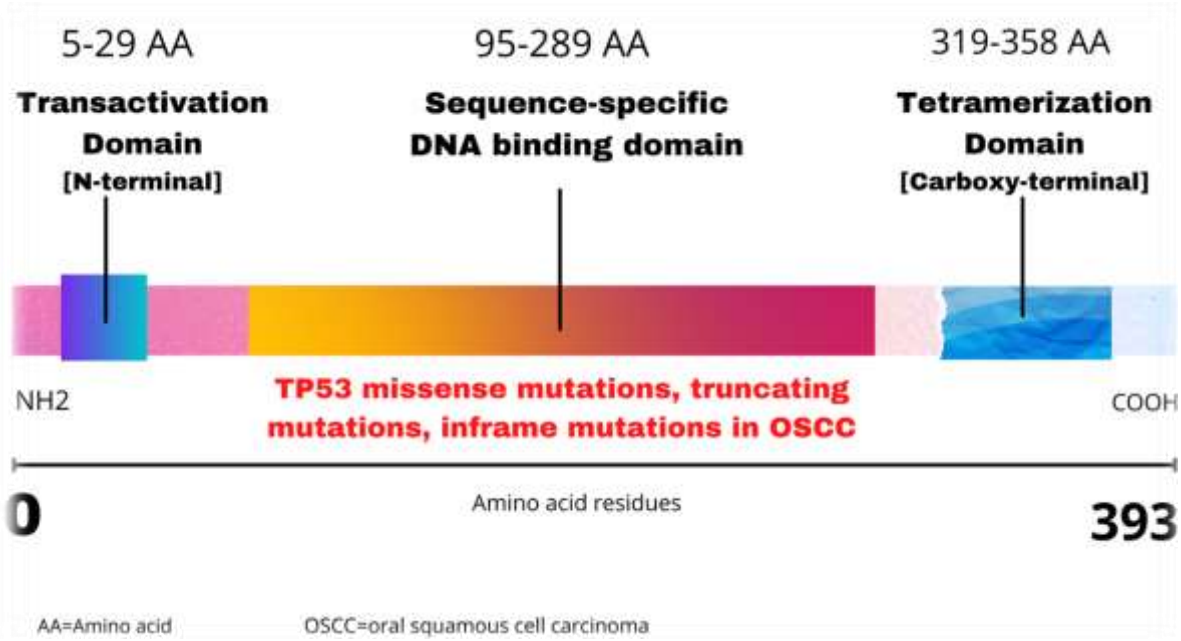

Image recreated from: 35; 42

Figure 3: The above diagram is illustrating the three major structural domains of P53: Sequence-specific DNA binding domain; tetramerization domain and transactivation domain. Most of the mutations in oral carcinoma have been detected in the DNA binding domain [95 to 289 amino acid residues] P53 codons. Missense T53 mutation is the most common form of mutation among OSCC patients.

A study on a population in Northern India consuming betel-quid and tobacco demonstrated substitution mutations in P53 genes, majorly missense and nonsense mutations in oral precancerous lesions and oral squamous cell carcinoma by polymerase chain reaction amplification of genomic DNA and direct sequencing. Mutations were noticed at codons 126, 136 and 174. These malignant lesions due to P53 missense mutation showed increased accumulation of P53 protein and circulating P53 antibodies **(**50**)**. The findings were highly comparable with the previous studies (32,49,35) which also highlighted different kinds of TP53 disruptive mutations in tobacco-induced OSCC.

Two types of TP53 disruptive mutations were identified in these research studies which are the truncating mutations and DNA binding domain missense [DBD] mutation (50, 32). The truncating mutations cause loss of the tumour-suppressive activity which results in an unfavourable prognosis. On the other hand, DBD mutation induces cell invasion; uncontrolled proliferation; metastasis and chemotherapy resistance. Lindemann et al also reported similar findings. Most of the missense mutations were found in the DNA binding domain. These mutations block binding of the wild type P53 gene to the transcription response elements and the transactivating downstream target genes inhibiting transactivation **(**35**)**. Further study is required to confirm these mutations **(**32l**)**. The molecular mechanisms involved in truncating mechanisms had not been explored in this study which needs further research.

Analysis of oral carcinoma cell lines revealed that P53 mutations are responsible for reduced survival rates in patients. Hence, early detection of P53 mutation is critical in predicting the generic changes and clinicopathological modifications these mutations would be causing (49). This requires advanced biological tools which could analyze genomes and detect mutations indicative of the carcinogenetic transformation of normal cells. P53 mutation sites could be used as therapeutic targets which could be blocked by novel biological tools to inhibit malignant proliferation **(**49**)**. The genetic variation in the cancer cells is indicating the need of classifying patients into molecular subgroups according to the specific genetic configuration through genomic sequencing. This will open doors for appropriate determination of novel therapeutic therapies for patients with poor prognoses **(**49**)**.

P53 mutation is associated not only with oral cancer initiation but also with poor prognosis. The poor prognosis could be attributed to TP53 mutation which exhibits a distinct property called "gain-of-function" which changes DNA binding properties and protein-protein interaction of P53 **(**32**)**. This property of "gain of function" is exhibited by the mutated p53 gene, unlike wild-type P53. The oncogenic cell transformation in the epithelial cell is attributed to this gain of function which imparts the ability of uncontrolled differentiation invading surrounding tissues without any inhibitory action from the mutated P53 leading to tumour formation with distant metastasis observed in mice models (32).

TP53 missense mutation is likely to alter normal cellular functions. Due to the mutation; cells differentiate more aggressively without coordination. This mechanism could explain the cell invasiveness, propensity to metastasize resulting in a poor prognosis (32). This new mechanism also confers resistance to chemotherapy complicating treatment for example genomic sequence of patients demonstrating truncated type P53 mutations demonstrated resistance to an anticancer drug, cisplatin (35). So, TP53 leads to tumorigenesis through various molecular pathways through multiple genetic events which require further investigations (3). For the early detection of smoking associated oral cancer, it is important to comprehend the intricate molecular mechanisms involved in carcinogenesis.

Impact of smoking on P53 gene in oral mucosa:

Different regions of oral mucosa which are susceptible to tobacco carcinogens induced malignant transformation are buccal; lingual (tongue), gingival, retromolar trigone, floor of the mouth, alveolar, soft palate, hard palate and tonsils (76). It was reported based on the cell line analysis of primary tumors of smokers that the regions of the short arm of chromosome 17 containing TP53 are often deleted with point mutations in the remaining TP53 allele. Tobacco smoke contains innumerable toxic compounds which are carcinogens. Researchers have identified more than 60 carcinogens in tobacco smoke which include PAH (Polycyclic aromatic hydrocarbons) like N-nitrosamines/NNK (Nicotine derived nitrosamine ketone); BaP (Benzo alpha Pyrene); Dibenz[a,h]anthracene, 5-methylchrysene and NNN[N'-nitrosonornicotine] (20)

While smoking oral epithelial cells are exposed to high concentrations of toxic compounds from tobacco smoke. One study examined the buccal mucosa brush biopsies from the oral cavity of smokers and non-smokers and found 100 buccal genes with altered expression in the tissues of smokers indicating oncogenic mutation (57). It reflected the potential of tobacco to induce genetic mutation. However, this study did not reveal the genetic alteration in P53.

Chronic exposure of oral epithelial cells to tobacco increases the absorption of carcinogens on the mucosal surface. These carcinogens undergo metabolic activation to react with DNA forming DNA adducts (20). DNA adducts are the complexes formed by the chemical bonding of a segment of DNA with the active form of carcinogen. These DNA adducts produce mutations that could initiate carcinogenesis (20). These tobacco-induced mutations are evident in both cancerous and non-cancerous lung cells. However, limited data is available about the relation between DNA adducts and oral carcinoma. Tobacco carcinogens, particularly PAH and NHK cause G to T transversions, a mutational pattern usually seen also in the DNA adduct formation (1).

One significant study in Taiwan used PCR-single strand conformation polymorphism and genomic sequencing to map the conserved regions of P53 gene which are exons 5-9 in OSCC samples from 187 tobacco, alcohol and betel quid consuming patients (76). Approximately, half of the patients (91) exhibited P53 mutations at exons 5-9. G:C and A:T were the most predominant transversions in P53 mutations associated with tobacco induced OSCC in this study on Taiwanese (76). This study strongly concluded that tobacco carcinogens forming DNA adducts contribute significantly to P53 mutations in OSCC amongst Taiwanese and alcohol enhances the mutagenic effects of tobacco carcinogens (76). Different patterns of base-pairs change observed among 187 OSCC tumour samples in this study were GC to AT (most prevalent); GC to TA; TA to AT; AT to CG; AT to CG; AT to GC; deletion and insertion of the base pairs (least prevalent) (76). It was proposed that methylation and deamination caused by the tobacco carcinogens are responsible for GC to AT mutations at CpG dinucleotide (76).

Another study in Taiwan reported similar findings where P53 was considered the key molecule regulating the gene expressions responsible for oral carcinoma. OSCC tumour samples exhibited mutations in p53 exon 4, P53 intron 6 regions in patients who had habits of smoking and betel nut chewing. Missense and non-sense mutations were detected in the conserved mid-regions of the P53 gene (exons 5-9) at codons 61, 175, 177, 222, 255, 266, 273, 277 and 282 in betel quid associated OSCC in Taiwanese (77). However, these studies were conducted only in Taiwanese population. Therefore, more studies are required in different countries to generalize the research findings.

Hainaut et al reported that there are certain DNA adducts detected in the lung which cause DNA base transversions but further research is required to confirm the presence of these adducts in oral carcinoma. The author predicted the need for further study to detect DNA adducts causing transversions. It can be inferred that tobacco carcinogens cause G to T transversion mutations. Results indicated that DNA damage from tobacco carcinogen leads to P53 mutation **(**22**)**. Chronic exposure to carcinogenic cigarette smoke was found to initiate base pairs transversions [GC to TA] which leads to P53 mutation **(**9**)**. Based on the findings researchers suggested that carcinogens like PAH in cigarette smoke induce DNA adduct formation with the guanine residues in DNA resulting in GC to TA transversions in oral mucosa initiating malignant transformation (76).

Furthermore, the examination of autopsy-derived explants of bronchial epithelial tissues showed that the cigarette smoke condensates like BAP and PAH bind to the cellular DNA altering the cellular phenotype **(**20**)**. In an in vivo experiment, the non-tumorigenic bronchial epithelial cells grown in the deepithelialized rat tracheas showed neoplastic transformation when chronically exposed to the tobacco carcinogen NNK illustrating the deleterious effect of tobacco in carcinogenesis. Also, the cancer genome atlas revealed that the genome-wide mutational burden was significantly higher in patients who are either current or former smokers (20).

A genome-wide mutation density study revealed higher mutation amongst smokers in comparison to non-smokers **(**20**)**. A study compared and analyzed 40 buccal biopsies of the smoker to that of 40 non- smokers to evaluate the effect of cigarette smoke on the transcriptome **(**the whole RNA analysis**)** and revealed that the chemicals in the tobacco smoke altered the expression of numerous genes. This study found that 32 genes demonstrated overexpression while 9 showed reduced expression in the oral mucosa of smokers in comparison to the non-smokers. Another study reported similar findings and concluded that the toxic components in cigarette smoke alter the transcriptome exhibiting overexpression of oncogenic genes **(**56**)**. There were striking similarities in the patterns of genetic alteration between oral and bronchial mucosa after chronic exposure to smoking 8.**)**.

Not only Cigarette smoking but shisha smoking **(**waterpipe**)** is also a very popular form of tobacco smoking; exclusively in the Middle Eastern countries and its use is growing worldwide. Public health professionals are considering shisha as a global threat because shisha smoke contains 69 carcinogens and tumor promoters. To assess the causation of carcinogens the researcher analyzed 105 paraffin-embedded oral squamous cell carcinoma tissue sections collected from 52 smokers and 53 non-smoker participants. The majority of the samples from shisha smokers demonstrated positive staining for p53 expression. Notably, shisha smoke results in increased smoke exposure with greater levels of Carbon monoxide when compared to cigarette smoking because shisha produces both tobacco and charcoal smoke imposing a higher risk of carcinoma (68).

Zaid et al found a positive correlation between tobacco consumption and p53 mutation in the benign as well as malignant oral mucosal epithelium. P53 mutation is prevalent in all neoplastic growths suggesting that loss of function in the P53 gene is the principal mechanism in tumorigenesis. Histological examination of the oral mucosa in a shisha smoker revealed p53 positive mucosa with an increased number of proliferating cells. P53 positivity was present mainly at the basal and parabasal layers of the oral mucosal epithelium in a pre-malignant lesion while scattered in the epithelial layers of poorly differentiated carcinoma. Researchers observed that P53 mutation is an early genetic event in tumorigenesis **(**68**)**. Therefore, detection of P53 is indicative of an initial stage of oral carcinoma which is why P53 could be an effective biomarker for the early detection of OSCC.

Brennan et al sequenced the P53 gene to determine the correlation of P53 with head and neck carcinoma. This study conducted sequence analysis of p53 gene in 129 tumour samples collected from HNSCC positive patients. Statistically significant results of this study revealed that 42% of patients exhibited P53 mutation among which 58% had a medical history of smoking [p=0.001]. Interestingly, two patients with a history of alcohol consumption only without any smoking showed no P53 mutation in this study. However, patients consuming both tobacco and alcohol demonstrated the highest % of P53 mutation. This study highlighted the difference in the P53 mutational pattern in smokers and

non-smokers. This striking variation indicates significant role of the tobacco carcinogens in the process of genetic mutations responsible for carcinogenesis. Hence, tobacco compounds contribute significantly to the HNSCC development and progression at the molecular level. This study revealed the lack of data analyzing the molecular targets of cigarette smoke causing P53 mutation (9).

Brennan et al proposed a mechanism of how carcinogens cause base-pair alteration in DNA leading to loss of function. The study proposed that carcinogens cause methylation and deamination of cytosine by the cellular enzymatic process. Some authors proposed that alcohol causes oral mucosal injury which in turn increases the absorption of carcinogens present in cigarette smoke. It further explained that one of the carcinogens Benzo [alpha] pyrene in tobacco smoke causes GC to TA transversion in the P53 gene (9). GC to AT transversion has also been identified in oesophageal cancer due to this carcinogen which is composed of stratified squamous epithelium similar to the oral epithelium. The base-pair transversion is attributed to the toxic carcinogens of tobacco which could increase the frequency of P53 mutations. Researchers concluded that transversions by chronic carcinogen exposure results in P53 mutation. This substitution of a base at the sub-atomic level suggests the interaction of carcinogenic particles with genes increasing the frequency of mutations. Nevertheless, the study also reported that P53 mutations have also been detected in cancers without any history of smoking and alcohol intake which suggest further research is necessary to confirm these research findings **(**9**).** Nevertheless**,** the contribution of smoking in p53 alteration in the oral mucosa cannot be refuted based on the current research evidence. Therefore, restoring P53 function could be therapeuticin oral cancer treatment.

P53: Potential as a molecular interventional therapy in the management of oral carcinoma

The tumour-suppressing property of TP53 could be used as an effective prognostic molecular marker and interventional therapy to predict the clinical outcomes in OSCC patients and as novel treatment therapy **(**35; 13; 69**)**. Despite the wide range of surgical and radical treatments, the overall survival rate of oral carcinoma remains poor **(**10**)**. Cancer is a disease with acquired genetic mutation. Hence, appropriate gene therapy could be highly effective, particularly in the treatment of recurrent cancer**(**13**)**. With the advent of gene therapy instead of conventional drugs, scientists are conducting experiments to find new therapeutic targets improving the overall survival rate in cancer **(**69**)**. Literature mining analysis and therapeutic target database mining have recognized P53 as a potent therapeutic target for oral cancer because it regulates multiple cancer pathways **(**10**)**. Several studies

reported the beneficial effect of administering P53 as therapy with adenovirus or retrovirus viral vector in several cancer types including head and neck, lung, liver, breast and cervical **(**14; 41; 55; 59, 13**)**.

Detection of clonal-specific P53 mutations at the margins of tumour in radically resected HNSCC is a predictor of local recurrence **(**14**)**. P53 gene therapy aims to restore the anti-tumour functions of wild-type P53 geneusing a viral vector in HNSCC patients **(**13; 34**)**. In vivo and in vitro Studies **(**13; 69; 34**)** on recombinant P53 therapy have demonstrated tumour regression in recurrent cancer patients. Clinical trials on adenovirus mediated P53 gene therapy administered different types of recombinant P53 transgene such as Gendicine, Advexin, ONYX 015 and H 101 in HNSCC patients to change the cancer phenotype. Clinical trials of ONYX 015 included oral carcinoma patients **(**13**)**. Gendicine and Advexin used a replication Adp53 vector while ONYX 015 and H 101 used CRAdp53 vectors. Studies evaluated the clinical trial data and reported that these therapies are safe and indicated as an adjunct to chemotherapy in recurrent HNSCC including oral cancer patients. Also, no severe adverse events had been reported during the clinical trials **(**13, 43**).**

Clayman et al provided preliminary support that adenovirus-mediated wild type P53 gene transfer has beneficial effects and can be used as a novel interventional therapy in advanced HNSCC in addition to surgery to prevent recurrence (14). Phase 1 and 2 clinical trials were conducted to evaluate the therapeutic effect of recombinant P53 gene therapy in recurrent HNSCC. The Ad-P53 therapy demonstrated an inhibitory effect on the cell proliferation in the xenograft models of HNSCC and preventive actions on tumorigenesis in the mouse xenograft models in the pre-clinical phase. Therefore, Liu et al conducted a randomized controlled unmasked phase 2 clinical trial in Beijing, China to evaluate the therapeutic effect of this recombinant adenoviral human P53 [Rad-P53] gene therapy combined with radiotherapy to prevent the recurrence of radically resected advanced oral carcinoma (36). This interventional trial was conducted among two groups: 51 patients with tongue carcinoma **(**TCA**)** and 56 patients with gingival carcinoma **(**GCA**)** randomized in experimental and control groups. The experimental group was administered multipoint injections of Rad-P53 into the tumour margins at a dose of $1x10^{12}$ viral particles in addition to radiotherapy while the control group was treated only with radiotherapy **(**dose=60 Gy at 3 weeks post-surgery**)**.

The recurrent rates were statistically significantly higher in the control group which received only radiotherapy in comparison to the experimental group [*recurrent rates of33.3% in TCA and 30.8% in GCA in the control group in comparison to only 7.4% in TCA and 6.7% in GCA in the experimental group*]. Also, the overall recurrent rate was statistically significantly lower in the experimental group, which is only 7% compared to the four times **(**32%**)** higher recurrence in the control group. The 3-year overall survival rate and disease-free survival rate were also higher in the experimental group which received recombinant P53 therapy in comparison to the control group **(**36**)**.

Based on the above findings investigators of this trial concluded that wound surface injection with recombinant P53 therapy as an adjunct to radiotherapy is safe and effective in different types of oral carcinoma patients **(**30**)**. Further trials are required to determine the long-term efficacy of the recombinant P53 therapy in oral cancer. Studies from other countries would be beneficial to determine the therapeutic benefits of P53 as a target therapy. Evidence-based research will determine the scalability of this intervention. P53 gene therapy in the treatment of oral cancer is in its infancy stage and in coming days more innovative methods will be evaluated through randomized controlled trials for multiple applications of P53 as a therapeutic agent, prognostic marker, and in early detection of all the p53 mutated cancers.

Conclusion:

Multiple studies suggested that tobacco carcinogens play an important role in the inactivation of the P53 gene in oral carcinoma inhibiting all the regulatory functions of cell growth. Based on the above discussion this study emphasizes abstinence from tobacco and alcohol consumption to alleviate the exponential rise in tobacco-induced oral cancer every year worldwide. Millions of cancer cases could be prevented every year by the inhibition of deleterious oral habits such as smoking, alcohol intake, betel nut chewing. It can be deduced from the studies that these lifestyle modifications will prevent P53 mutation to a great extent.

This study explored the relationship of tobacco carcinogen, P53 mutation and oral carcinoma and concluded that tobacco carcinogens induce p53 mutation leading to the loss of control on abnormal cell growth. The highly intricate genetic events leading to uncontrolled cell differentiation in smoking-associated oral squamous cell carcinoma are difficult to contemplate from a molecular perspective. Further research is required to determine the domains and binding sites of P53 interacting with the tobacco carcinogens and rigorous research on the interaction of DNA adducts with oral mucosal cells causing p53 mutation. The literature review revealed that limited studies are available exploring the pathways triggering p53 mutation in smoking-associated oral carcinoma. Further study is required to understand the etiopathogenesis of oral carcinoma and pathways triggering P53 mutation.

This study revealed the significant role of amino acid arginine which is the most commonly mutated residue at DNA binding domain in cancers. Most studies have emphasized the impact of P53 mutation on the clinical outcomes of oral cancer patients in comparison to the molecular mechanisms of P53 mutations and their potential as a therapeutic agent in oral cancer treatment. Hence, further research is required to understand the molecular basis of tobacco carcinogen-induced oral carcinoma to identify unknown key biomarkers and genes which could be used as therapeutic targets for improved

management of oral cancer in the future. This study proposes to conduct further clinical trials to explore the therapeutic potential of P53 as an anti-carcinogenic agent. Further research is advised on the DNA binding domain of P53 to detect amino acid residues involved in mutations in relation to oral carcinoma.

This study will provide a stimulus for further studies emphasizing the molecular mechanisms of tobacco carcinogens causing P53 mutation in oral carcinoma. Also, this study will act as a medium to convey the message that smoking is not just a bad oral habit but an appalling risk factor for oral squamous cell carcinoma. Every component in tobacco **(**smoke and smokeless**)** is a risk factor giving rise to numerous cancers. It has the potential to change the genotype of a cell completely inactivating physiological functions. Hence more public health interventions must be implemented across the globe to raise awareness among people about the negative impact of smoking on human genomics.

Conflict of interest: None

Acknowledgment:

I am thankful to my parents, aunt Farah and professors Jennie Day and Kate Jehan who taught me the essentials of scientific writing for their constant support and encouragement.

References

1.Agarwal, Varun Kumar, et al. “Clinical and Histopathological Correlation of P16 and P53 Expression in Oral Cancer.” Indian Journal of Surgical Oncology, 2020, doi:10.1007/s13193-020-01145-z.

2.Atrens, Dale M. "Nicotine as an addictive substance: a critical examination of the basic concepts and empirical evidence." Journal of Drug Issues 31.2 (2001): 325-394.

3.Ali, Johar, et al. “Genetic Etiology of Oral Cancer.” Oral Oncology, vol. 70, 2017, doi:10.1016/j.oraloncology.2017.05.004.

4. Baak, J. P. A., et al. "Genomics and proteomics in cancer." *European Journal of Cancer* 39.9 (2003): 1199-1215.

5. Baral, Rathindranath, Srinivas Patnaik, and Bibhu Ranjan Das. "Co-overexpression of p53 and c-myc proteins linked with advanced stages of betel-and tobacco-related oral squamous cell carcinomas from eastern India." European journal of oral sciences 106.5 (1998): 907-913.

6. Bailar, John C., and Heather L. Gornik. "Cancer undefeated." New England Journal of Medicine 336.22 (1997): 1569-1574.

7. Berg, Sarah A., et al. "Nicotine is more addictive, not more cognitively therapeutic in a neurodevelopmental model of schizophrenia produced by neonatal ventral hippocampal lesions." Addiction biology 19.6 (2014): 1020-1031.

8. Benowitz, Neal L., and Jack E. Henningfield. "Reducing the nicotine content to make cigarettes less addictive." Tobacco control 22.suppl 1 (2013): i14-i17.

9. Boyle, Jay O., et al. “Effects of Cigarette Smoke on the Human Oral Mucosal Transcriptome.” Cancer Prevention Research, vol. 3, no. 3, 2010, doi:10.1158/1940-6207.CAPR-09-0192.

10. Brennan, Joseph A., et al. “Association between Cigarette Smoking and Mutation of the P53 Gene in Squamous-Cell Carcinoma of the Head and Neck.” New England Journal of Medicine, vol. 332, no. 11, 1995, doi:10.1056/nejm199503163321104.

11. Bundela, Saurabh, et al. “Potential Therapeutic Targets for Oral Cancer: ADM, TP53, EGFR, LYN, CTLA4, SKIL, CTGF, CD70.” PLoS ONE, vol. 9, no. 7, 2014, doi:10.1371/journal.pone.0102610.

12. Chakrobarty, Bidyut, et al. "Relationship among tobacco habits, human papilloma virus (HPV) infection, p53 polymorphism/mutation and the risk of oral squamous cell

13. carcinoma." Journal of oral and maxillofacial pathology: JOMFP 18.2 (2014): 211.

14. Chen, Guang-xia, et al. "Clinical utility of recombinant adenoviral human p53 gene therapy: current perspectives." OncoTargets and therapy 7 (2014): 1901.

15. Clayman, Gary L., et al. “Adenovirus-Mediated Wild-Type P53 Gene Transfer as a Surgical Adjuvant in Advanced Head and Neck Cancers.” Clinical Cancer Research, vol. 5, no. 7, 1999.

16. Chung, Christine H., et al. "Genomics and proteomics: emerging technologies in clinical cancer research." *Critical reviews in oncology/hematology* 61.1 (2007): 1-25.

17. D'amico, D., et al. "High frequency of somatically acquired p53 mutations in small-cell lung cancer cell lines and tumors." Oncogene 7.2 (1992): 339-346.

18. Efeyan, Alejo, and Manuel Serrano. "p53: guardian of the genome and policeman of the oncogenes." Cell cycle 6.9 (2007): 1006-1010.

19. Forbes, Simon A., et al. "COSMIC (the Catalogue of Somatic Mutations in Cancer): a resource to investigate acquired mutations in human cancer." Nucleic acids research 38.suppl_1 (2010): D652-D657.

20. Gibbons, Don L., et al. “Smoking, P53 Mutation, and Lung Cancer.” Molecular Cancer Research, vol. 12, no. 1, 2014, doi:10.1158/1541-7786.MCR-13-0539.

21. Granville, Courtney A., and Phillip A. Dennis. "An overview of lung cancer genomics and proteomics." American journal of respiratory cell and molecular biology 32.3 (2005): 169-176.

22. Goodsell, D. S. “P53 Tumor Suppressor.” RCSB Protein Data Bank, July 2002, doi:10.2210/rcsb_pdb/mom_2002_7.

23. Gupta, Neha, et al. “Changing Trends in Oral Cancer – a Global Scenario.” Nepal Journal of Epidemiology, vol. 6, no. 4, 2017, doi:10.3126/nje.v6i4.17255.

24. Hainaut, Pierre, and Gerd P. Pfeifer. “Patterns of P53→T Transversions in Lung Cancers Reflect the Primary Mutagenic Signature of DNA-Damage by Tobacco Smoke.” Carcinogenesis, vol. 22, no. 3, 2001, pp. 367–74, doi:10.1093/carcin/22.3.367.

25. Halazonetis, Thanos D., and A. N. Kandil. "Conformational shifts propagate from the oligomerization domain of p53 to its tetrameric DNA binding domain and restore DNA binding to select p53 mutants." The EMBO journal 12.13 (1993): 5057-5064

26. Hecht, Stephen S. “Tobacco Carcinogens, Their Biomarkers and Tobacco-Induced Cancer.” Nature Reviews Cancer, vol. 3, no. 10, 2003, doi:10.1038/nrc1190.

27. Hafsi, Hind, et al. "Effects of Δ40p53, an isoform of p53 lacking the N-terminus, on transactivation capacity of the tumor suppressor protein p53." BMC cancer 13.1 (2013): 1-10.

28. Jain, Abhinav K., and Michelle Craig Barton. "Making sense of ubiquitin ligases that regulate p53." Cancer biology & therapy 10.7 (2010): 665-672.

29. Jiang, Xiaoge, et al. “Tobacco and Oral Squamous Cell Carcinoma: A Review of Carcinogenic Pathways.” Tobacco Induced Diseases, vol. 17, no. April, 2019, doi:10.18332/tid/105844.

30. Jones, Peter A., and Stephen B. Baylin. "The epigenomics of cancer." Cell 128.4 (2007): 683-692.

31. Jurel, Sunit Kumar, et al. "Genes and oral cancer." *Indian journal of human genetics* 20.1 (2014): 4.

32. Kaur, Jasbir, Anurag Srivastava, and Ranju Ralhan. "Overexpression of p53 protein in betel-and tobacco-related human oral dysplasia and squamous-cell carcinoma in India." International journal of cancer 58.3 (1994): 340-345.

33. Kerdpon, Duangporn, HutchaSriplung, and SuparpKietthubthew. "Expression of p53 in oral squamous cell carcinoma and its association with risk habits in southern Thailand." Oral oncology 37.7 (2001): 553-557.

34. Krishna, Akhilesh, et al. “Molecular Concept in Human Oral Cancer.” National Journal of Maxillofacial Surgery, vol. 6, no. 1, 2015, doi:10.4103/0975-5950.168235.

35. Lapke, Nina, et al. “Missense Mutations in the TP53 DNA-Binding Domain Predict Outcomes in Patients with Advanced Oral Cavity Squamous Cell Carcinoma.” Oncotarget, vol. 7, no. 28, 2016, doi:10.18632/oncotarget.9925.

36. Lerman, Caryn, and Wade Berrettini. "Elucidating the role of genetic factors in smoking behavior and nicotine dependence." American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 118.1 (2003): 48-54.

37. Li, Yi, et al. "Key points of basic theories and clinical practice in rAd-p53 (Gendicine™) gene therapy for solid malignant tumors." Expert opinion on biological therapy 15.3 (2015): 437-454.

38. Lindemann, A., et al. “Targeting the DNA Damage Response in OSCC with TP53 Mutations.” Journal of Dental Research, vol. 97, no. 6, 2018, doi:10.1177/0022034518759068.

39. Liu, S., et al. “Randomized, Controlled Phase II Study of Post-Surgery Radiotherapy Combined with Recombinant Adenoviral Human P53 Gene Therapy in Treatment of Oral Cancer.” Cancer Gene Therapy, vol. 20, no. 6, 2013, doi:10.1038/cgt.2013.30.

40. Loukola, Anu, et al. "Genetics and smoking." Current addiction reports 1.1 (2014): 75-82.

41. Makarev, Eugene, et al. "In silico analysis of pathways activation landscape in oral squamous cell carcinoma and oral leukoplakia." *Cell death discovery* 3.1 (2017): 1-7.

42. Morse, Douglas E., et al. "Smoking and drinking in relation to oral cancer and oral epithelial dysplasia." Cancer Causes & Control 18.9 (2007): 919-929.

43. Mujib, Sahudi Abdul, et al. "Cell Death and Induced P53 Expression in Oral Cancer, HeLa, and Bone Marrow Mesenchyme Cells under the Exposure to Noncontact Electric Fields." Integrative Medicine International, vol. 4, no. 3–4, 2017, doi:10.1159/000485186.

44. Navin, Nicholas E. "The First Five Years of Single-Cell Cancer Genomics and Beyond." Genome Research, vol. 25, no. 10, 2015, pp. 1499–507, doi:10.1101/gr.191098.115.

45. Nemunaitis, J., et al. "Adenovirus-mediated p53 gene transfer in sequence with cisplatin to tumors of patients with non–small-cell lung cancer." Journal of Clinical Oncology 18.3 (2000): 609-609.

46. Pavletich, Nikola P., Kristen A. Chambers, and Carl O. Pabo. "The DNA-binding domain of p53 contains the four conserved regions and the major mutation hot spots." Genes & development 7.12b (1993): 2556-2564.

47. Pearson, Sue, Hepeng Jia, and Keiko Kandachi. "China approves first gene therapy." (2004): 3-4.

48. Pfeifer, Gerd P., et al. "Tobacco Smoke Carcinogens, DNA Damage and P53 Mutations in Smoking-Associated Cancers." Oncogene, vol. 21–48, no. 6, 2002, doi:10.1038/sj.onc.1205803.

49. Petti, Stefano. "Lifestyle risk factors for oral cancer." Oral oncology 45.4-5 (2009): 340-350.

50. Pontieri, Francesco E., et al. "Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs." Nature 382.6588 (1996): 255-257.

51. Powell, Darren J., et al. "Stress-dependent changes in the properties of p53 complexes by the alternative translation product p53/47." Cell Cycle 7.7 (2008): 950-959.

52. Protein Data Bank [PDB], "Molecule of the month, P53 Tumor suppressor", 2002, Available at pdb101.rcsb.org/motm/31 [Accessed on 27th February 2021].

53. Rahman, Mahbubur, Junichi Sakamoto, and Tsuguya Fukui. "Bidi smoking and oral cancer: ameta-analysis." International journal of cancer 106.4 (2003): 600-604.

54. Ragos, Vasileios, et al. “P53 Mutations in Oral Cavity Carcinoma.” Journal of B.U.ON., vol. 23, no. 6, 2018, pp. 1569–72.

55. Ralhan, R., et al. “Correlation between P53 Gene Mutations and Circulating Antibodies in Betel- and Tobacco-Consuming North Indian Population.” Oral Oncology, vol. 37, no. 3, 2001, doi:10.1016/S1368-8375(00)00092-0.

56. Sadri, GhH, and H. Mahjub. "Tobacco smoking and oral cancer: a meta-analysis." Journal of research in health sciences 7.1 (2007): 18-23.

57. Shah, Franky D., et al. "A review on salivary genomics and proteomics biomarkers in oral cancer." Indian Journal of Clinical Biochemistry 26.4 (2011): 326-334.

58. Sandoval, Juan, and Manel Esteller. "Cancer epigenomics: beyond genomics." Current opinion in genetics & development 22.1 (2012): 50-55.

59. Sathish Kumar, M., et al. “Gene Therapy in Oral Cancer: A Review.” Journal of Clinical and Diagnostic Research, vol. 7, no. 6, 2013, doi:10.7860/JCDR/2013/5412.3071.

60. Schuler, Martin, et al. "A phase I study of adenovirus-mediated wild-type p53 gene transfer in patients with advanced non-small cell lung cancer." Human gene therapy 9.14 (1998): 2075-2082.

61. Serafini, Mara S., et al. "Transcriptomics and Epigenomics in head and neck cancer: Available repositories and molecular signatures." Cancers of the head & neck 5.1 (2020): 1-10.

62. Sridhar, Sriram, et al. “Smoking-Induced Gene Expression Changes in the Bronchial Airway Are Reflected in Nasal and Buccal Epithelium.” BMC Genomics, vol. 9, 2008, doi:10.1186/1471-2164-9-259.

63. Sung, Hyuna, et al. "Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries." CA: A Cancer Journal for Clinicians (2021).

64. Swisher, Stephen G., et al. "Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer." Journal of the National Cancer Institute 91.9 (1999): 763-771.

65. Urashima, Mitsuyoshi, et al. "Distinct effects of alcohol consumption and smoking on genetic alterations in head and neck carcinoma." PloS one 8.11 (2013): e80828.

66. Viet, Chi T., and Brian L. Schmidt. "Understanding oral cancer in the genome era." Head & neck 32.9 (2010): 1246-1268.

67. Vincent-Chong, Vui King, et al. "Genome Wide Profiling in Oral Squamous Cell Carcinoma Identifies a Four Genetic Marker Signature of Prognostic Significance." PLoS ONE, vol. 12, no. 4, 2017, doi:10.1371/journal.pone.0174865.

68. Vogelstein, Bert, David Lane, and Arnold J. Levine. "Surfing the p53 network." Nature 408.6810 (2000): 307-310.

69. Walsh, Tom, and Mary-Claire King. "Ten genes for inherited breast cancer." Cancer cell 11.2 (2007): 103-105.

70. Wong, Yong-Kie, et al. "p53 alterations in betel quid-and tobacco-associated oral squamous cell carcinomas from Taiwan." Journal of oral pathology & medicine 27.6 (1998): 243-248.

71. World Health Organisation [WHO]. "Global data on incidence of oral cancer (maps)", 2005, Available at www.who.int/oral_health/publications/oral_cancer_brochure.pdf?ua=1 [Accessed on 27th February 2021].

72. Xu, Jie, et al. "Alterations of p53, cyclin D1, rb, and H-ras in human oral carcinomas related to tobacco use." Cancer: Interdisciplinary International Journal of the American Cancer Society 83.2 (1998): 204-212.

73. Yauk, Carole L., et al. "Mainstream tobacco smoke causes paternal germ-line DNA mutation." Cancer research 67.11 (2007): 5103-5106.

74. Zaid, Khaled, et al. "P53 Overexpression in Oral Mucosa in Relation to Shisha Smoking in Syria and Lebanon." Asian Pacific Journal of Cancer Prevention, vol. 19, no. 7, 2018, doi:10.22034/APJCP.2018.19.7.1879.

75. Zhang, S. W., et al. "Recombinant adenovirus-p53 gene therapy combined with radiotherapy for head and neck squamous-cell carcinoma." Zhonghuazhongliu za zhi [Chinese journal of oncology] 27.7 (2005): 426-428.

76. Hsieh, Ling-Ling, et al. "Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese." *Carcinogenesis* 22.9 (2001): 1497-1503.

77. Chen, Yin-Ju, et al. "Head and neck cancer in the betel quid chewing area: recent advances in molecular carcinogenesis." *Cancer science* 99.8 (2008): 1507-1514.

Volume 2 Issue 5 May 2021