

p53: The Guardian of Genome Against OSCC Progression

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ABSTRACT: *Protein 53 (p53) plays a vital role in the human body, mainly as tumor suppressor protein, by inhibiting tumor cell formation and DNA damage through gene target regulation. Known as "guardian of genome", p53 protects genome integrity and prevents cells' proliferation with damaged DNA. In oral squamous cell carcinoma, there is approximately 85% mutation of p53, which leads to impairment of its normal function and often correlated with OSCC progression. Those findings signify the role of p53 in guarding against OSCC, which are through cell cycle arrest, DNA repair, and apoptosis.*

Keyword: *cell cycle arrest, OSCC, p53*

1. INTRODUCTION

Protein 53 (p53) is an important tumor suppressor protein, which plays an important role, both physiologically or pathologically. It acts its primary function by inhibiting tumor cell formation and DNA damage through gene target regulation, whose role in cell cycle arrest, DNA repair, or apoptosis [1,2]. Known as "guardian of genome", p53 protects genome stability and prevents the proliferation of cells with damaged DNA, thus preventing tumorigenesis [3].

Oral squamous cell carcinoma (OSCC) is a malignancy found in the epithelial of the oral cavity, lips, salivary glands [7]. OSCC has a relatively high death rate, with a five-year survival rate of less than 50% [8]. Despite the advancement of treatment and technology in the last 30 years, there is no significant improvement in its five-year survival rate [9].

Many research reported a high incidence of p53 mutation in OSCC, up to 85% [10–12]. Mutation of p53 can impair p53 function as a tumor suppressor, thus lead to tumor formation in OSCC [13], thus signify the importance of normal p53 as a guardian against OSCC

progression. This narrative literature review will discuss more about OSCC, p53 and the exact role of p53 in preventing tumor formation in OSCC.

2. ORAL SQUAMOUS CELL CARCINOMA

Oral cancer is a specific malignancy found in the oral cavity, lips, salivary glands [7,14]. Oral Squamous Cell Carcinoma (OSCC) is the dominant manifestation of oral cancer as 90% of oral cancer is histologically originated from squamous cell epithelial [7]. OSCC also a part of head and neck squamous cell carcinoma, the sixth higher incidence of malignancies globally, with 400.000 new cases recorded each year [11]. OSCC has several stages and has metastatic tendencies to lymph nodes [7].

OSCC has a relatively high death risk with a five-year survival rate of less than 50% [8]. Despite the advancement of cancer drug and treatment to elevate the survival rate of OSCC patients, generally, there is no significant improvement in the survival rate of OSCC patient in the last 30 years [9,15]

Risk Factors of OSCC

OSCC is often associated with various risk factors such as the living environment and specific lifestyles such as alcohol consumption, tobacco smoking, and ultraviolet (UV) light exposure [16]. More than 75% of OSCC cases are correlated with tobacco smoking and alcohol consumption, in which smoking could increase the OSCC incidence by ten folds. Simultaneously, the combination of both can result in more unsatisfactory outcomes and progressivity [17]. Several virus strains such as *human papillomavirus* (HPV) and *Epstein–Barr virus* (EBV) were reported as the major risk factors. In addition, genetic factors, lack of nutrition, betel chewing, poor oral health, and hygiene are also risk factors for OSCC [18,19]. Those risk factors can cause an abnormal response to DNA damages, which lead to cell death, chromosome instability, and uncontrolled proliferation [16].

Molecular Pathogenesis of OSCC

In general, malignancy development happens through several stages, starting from pre-malignancies lesion to invasive malignancies with clinical metastasis. This condition is supported by the accumulation of various molecular changes which increase the malignancy potential. A similar progression is found in OSCC, which started from benign hyperplasia, dysplasia, in situ carcinoma, and lastly, it becomes the invasive carcinoma followed by genomic changes [20].

The effect of smoking, alcohol consumption, or other carcinogens results in the initial changes, a deactivation or gene mutation of TP53 and RB1, which consecutively code the tumor suppression protein, p53 retinoblastoma (pRb). These changes cause the functional impairment and level decrease of both proteins [21]. These conditions usually followed by the mutation of pro-oncogene genes such as Epithelial Growth Factor Receptor (EGFR) and MDM2, causing the overexpression of those proteins. EGFR has an essential role in promoting epithelial proliferation, while MDM2 can bind p53 to form a complex and impair the activity of p53 and initiate the degradation of p53 through its role as a ubiquitin E3 ligase for p53 [22]. Combining the decreased level of p53 and pRB and the increased level of EGFR and MDM2 resulted in uncontrolled proliferation. A slight different mechanism happened to

OSCC caused by HPV, as the pathogenesis is initiated by the synthesis of cell cycle regulator in the genomic region of HPV, namely E6, which bind and induce the degradation of p53 and E7 inhibit the activity of pRB [23]. In this stage, the cancer cell with dysplasia is proliferating, and clinically it will be found as hyperplasia or mild dysplasia [20].

When the condition gets worsens, the mutation of the CDKN2A gene happens. This mutation can lead to the decrease of p16 expression, a tumor suppressor protein whose role in inhibit the cyclin D1-CDK4 complex, increasing cyclin D expression and cause the G1 phase transition to the S phase [24]. For the nutritional support for cancer cells, VEGF and VEGFR are stimulated for angiogenesis purposes, which will aggravate cancer cell progression. In this stage, the dysplasia will worsen, and through histopathological examination, it will be diagnosed as severe dysplasia or, worst, OSCC *in situ* [20].

From OSCC *in situ* to invasive OSCC, the cancer cell needs to pass the epithelial junction barrier. In this stage, the stimulation of matrix metalloproteinases (MMP) plays a significant role. MMP is a proteolytic enzyme group capable of degrading extracellular matrices, and it holds a vital role in tumor progressions such as tumor invasion, angiogenesis, and metastasis [25]. The increase of MMP-2 and MMP-9 levels is observed in this stage and leads to matrix degradation, allowing the OSCC cell invasion or metastasis. [20].

3. p53 PROTEIN

As previously stated in the pathogenesis of OSCC, p53 plays a vital role in the initial stage of OSCC progression. p53 is a tumor suppressor protein, which has an important role in both physiologically or pathologically. However, its primary role is inhibiting tumor cell formation and DNA damage through gene target regulation, whose role in cell cycle arrest, DNA repair, or apoptosis [1,2]. These crucial roles of p53 give this gene another name, which is "guardian of genome" [2].

p53 is coded by TP53 gene located in chromosome 17p13 [26,27]. In the normal condition, p53 has a very short half-life and is maintained at a very low level in the tissue by MDM2, the E3 ubiquitin ligase of p53 [12]. In contrast, in the cellular stress condition due to DNA damage, hypoxia, and oncogene activation, the level of p53 in the tissue will increase, and the protein will be activated. The activated p53 can function as a transcription factor to a specific gene, which plays a role in cell cycle arrest, promotes cell senescence, or induces the cell to apoptosis pathway [12,28].

p53 Structure

The structure of p53 consists of 393 amino acid residues in the form of homotetramer, with 43kDa of molecular weights [26,29]. Six main parts structured this protein (Figure 1), initiated by unfolded *amino (N) - terminal transactivation domain* (TAD), which divided into two sub-domains, TAD1 and TAD2. TAD1 is functioning as the primary binding site of MDM2 and MDMX. TAD2 has a role in the activation of transcription and protein interaction with RPA. Thus, TAD1 and TAD 2 is an essential part of p53 for its function as a tumor suppressor and p53 degradation and activity during cellular stress condition [30].

TAD1 and TAD2 are followed by a proline-rich region (PRR), which is essential in maintaining the stability of p53 through Pin1 binding [26,31]. PRR also mediates binding to

cofactors essential for efficient transcription [31]. This PRR connects TAD to the core domain (CD) or DNA binding domain (DBD), the functional domain in p53 responsible for binding the specific sequence of DNA target. DBD consists of various arginine amino acids with one zinc atom (5'-D(CPGPGPGPCPAPTGPCCPCPG)-3'), which interact with the DNA molecule [26,32,33].

DBD is connected by flexible linker (L) region to the oligomerization domain (OD), a domain responsible for tetramerization, which is important for p53 activity. The structure ends in the regulatory domain of extreme carboxyl terminus or carboxyl terminus domain (CTD) [6,32,34]. CTD plays a vital role in down-regulating DBD activity [33].



Figure1. The structure of p53 [33]

p53 Function

There are various essential roles of p53 in the human body. However, the primary function of this protein is to prevent the proliferation of cell with DNA damage by controlling and monitoring the cell cycle [26,28]. The p53 protein is activated by various cellular stressors, such as DNA damage, hypoxia, and oncogene activity [12]. After the activation, p53 will generally function as a transcription factor in a specific sequence to arrest the cell cycle, promote the cell senescence, or induce the apoptosis to damaged cells, thus prevent the proliferation of the damaged cells [12,28,35,36].

The damage that occurs in the double helix of DNA incited by ion radiation will activate ataxia telangiectasia mutated (ATM) kinase to phosphorylate MDM2, the ubiquitin protein of p53. This condition allows p53 to increase in the tissue and forms the tetramer with other p53 subunits, thus lead to the activation of p53 [37]. The active p53 will function as transcription factor of specific gene by binding to specific target sequence of hundreds genes, which play important roles in cell cycle arrest, cell senescence, cell apoptosis, metabolism, and differentiation, thus prevent cell with damaged DNA to proliferate and prevent the tumor formation [12,28,35,36]. The arrest in the cell cycle is reversible and has a vital role in maintaining chromosome integrity by giving cells time to repair the DNA damage [36].

4. p53 ROLE AGAINST OSCC PROGRESSION

Guarding Through Cell Cycle Arrest

As described in the pathogenesis of OSCC, tumor formation is initiated by cellular stress, such as carcinogens exposure, which leads to DNA damage. When DNA damage happens, the TP53 gene is activated, which initiating p53 protein transcription. ATM is activated and stimulates MDM2 phosphorylation, allowing the increase of p53 level in the tissue [36]. Active p53 starts its function as guardian of the genome by firstly arrest the cell cycle to prevent the replication of cells with damaged DNA through activating the transcriptional target gene of p21, which is widely known to play an essential role in cell

cycle arrest. p21 stimulates cell cycle arrest by inhibiting P21 cyclin-dependent kinase (cyclin-CDK) complex formation to provide appropriate time for DNA to repair [6]. The continuation of the cell cycle from one to another phase depends on the cyclin-CDK complex, which plays as both regulator and catalyst for the cell cycle. There are four phases in the cell cycle, and each phase has its cyclin-CDK complex [38]. As cyclin-dependent kinase inhibitor, p21 can bind to CDK2-cyclin E in the late G phase, to CDK2-cyclin A in the late S phase, to CDK1-cyclin A in the G2 phase, and to CDK1-cyclin B in the M phase, thus allowing the cell cycle to arrest [23,39]. Cell cycle arrest is reversible, consequently, after DNA damage is successfully repaired, the arrest period will end, and the cycle continues normally. However, chronic activation of p53 and prolonged p21 overexpression can lead to irreversible cell cycle arrest and lead to cell senescence [36].

Guarding Through DNA Repair

The period graced due to cell cycle arrest bring enough time for DNA repair process. Although not directly, p53 also plays a role in DNA repair through its involvement in global genome Nucleotide Excision Repair (GG-NER) pathway regulation, which is essential for the repair process of DNA damage due to UV irradiation. p53 is also involved in regulating Base Excision Repair (BER) activities in the cell cycle [40].

Guarding Through Apoptosis

When the damaged area is immense, p53 will induce cell apoptosis through both intrinsic and extrinsic pathways [41]. The activation of extrinsic pathways happens through the stimulation and dimerization of death receptors (Fas and Dr5), which activate procaspase 8 and resulting in the activation of caspase 3 and 7. The activation of intrinsic pathway occurs through mitochondrial outer membrane permeabilization (MOMP), which decided by pro-apoptosis protein (ie: p53 upregulated modulator of apoptosis (PUMA), Noxa) and antiapoptosis protein (ie: B-cell lymphoma 2 (Bcl2), B-cell lymphoma-extra large (BclXL), Mcl1). The direct binding of p53 to Bcl2 and BclXL will impair their function and stimulate PUMA and Noxa. Both PUMA and Noxa can activate and insert Bcl2 Associated X (Bax) and Bcl2 homologous antagonist killer (Bak) through the outer membrane of mitochondria and form the pores in the membrane, which lead to cytochrome c release. The release cytochrome c will bind to adenosine triphosphate (ATP) and apoptotic protease activating factor (Apaf1), resulting in apoptosome complex formation, which activate procaspase 9. The activation of procaspase 9 will stimulate caspase 3 and 7, thus induce apoptosis [36].

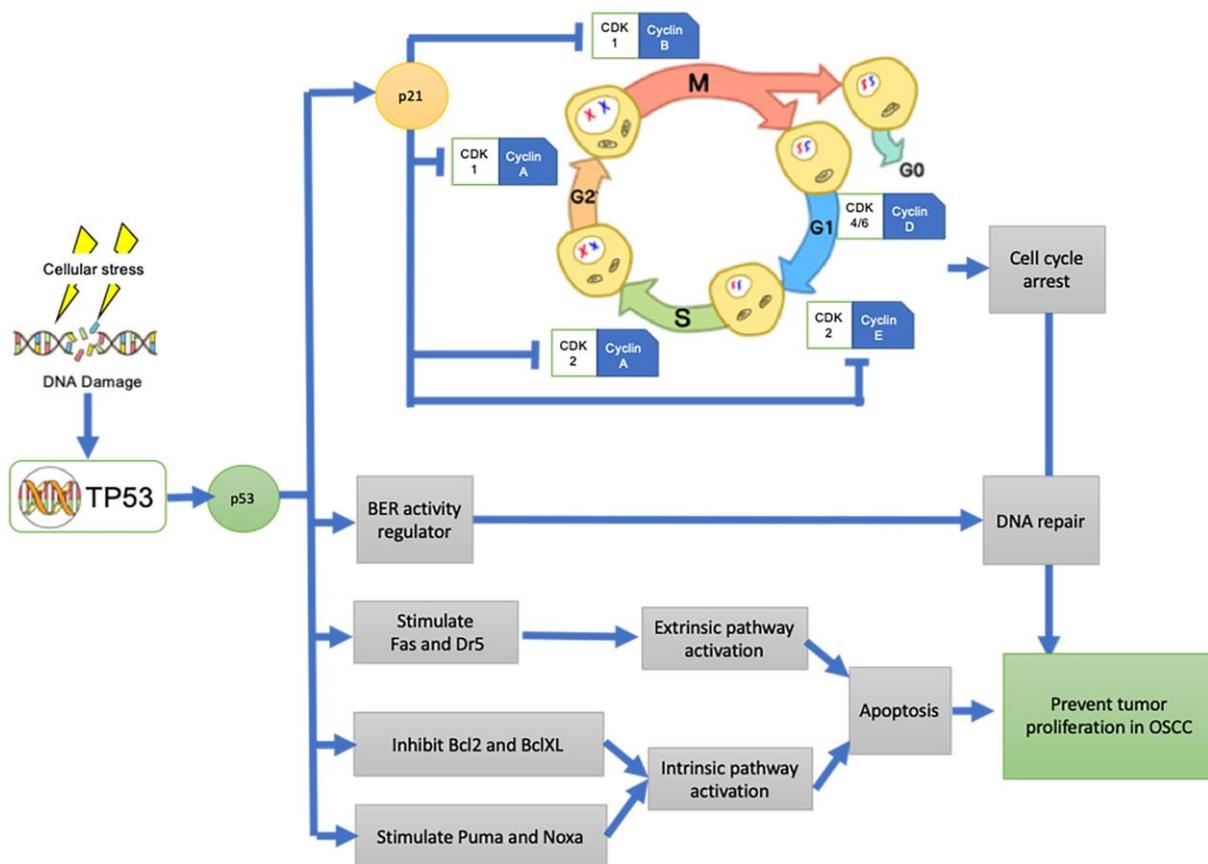


Figure 2. The role p53 against OSCC

All these activities of p53 (Figure 2) synergistically inhibit the proliferation of damaged cells and prevent tumor formation. In the case of OSCC, with this prevention measure of p53, why tumor proliferation still happens? As previously described, OSCC is a multifactorial disease; there are many risk factors for OSCC to happen. OSCC possibly happens due to the damaged area is immense and exceeded its ability to repair. Thus, tumor formation cannot be prevented. Another possibility is the mutation of p53, which will impair its normal function [33].

There is a 65-85% mutation of p53 protein in OSCC cases [10]. Mutant p53 will lose its function as a tumor suppressor, which is often called loss of function (LOF) [11,42]. In addition, mutant p53 can gain function (GOF) as pro-oncogene, thus promoting tumorigenesis and aggravating cancer progression clinically [12]. Mutant p53 can also inactivate the normally functioning wild-type p53 by its dominant-negative activity through oligomerization [33]. Overexpression of mutant p53 in OSCC is also correlated to its resistance to several chemotherapy drugs such as cisplatin, alkylating agents (temozolomide), anthracyclines (doxorubicin), antimetabolites (gemcitabine), antiestrogens (tamoxifen) and EGFR-inhibitors (cetuximab) [2]. Recent studies also showed that mutation in five hotspot codons of TP53 decreased the sensitivity of cisplatin-based chemotherapy and resulting in more worse outcome clinically [5,43]. Therefore, the mutational status of p53 can

play an essential role in deciding the prognosis and the appropriate treatment for OSCC in the future.

5. CONCLUSION

As tumor suppressor protein, p53 can prevent the OSCC formation by arresting the cell cycle through p21, regulating BER activity for DNA repair, and inducing apoptosis by activating the intrinsic and extrinsic pathways.

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