ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Promoter Hypermethylation of p15 Tumor Suppressor Gene in OSCC Among North Indian Population.

Saumya Singh, Richa Kumari, Siddharth Singh, Madhav Kumar, Abhimanyu Kumar Jha*.

Department of Biotechnology, Sharda School of Engineering and Technology, Sharda University, Greater Noida, India, 201310.

ABSTRACT

Oral Squamous Cell Carcinoma poses a significant health burden globally. This study delves into the epigenetic mechanisms underlying Oral Squamous Cell Carcinoma pathogenesis, focusing on the methylation status of the p15 gene promoter. Through analysis of DNA samples extracted from Oral Squamous Cell Carcinoma patients and controls, we elucidate the role of p15 in cell cycle regulation and its dysregulation in disease states. Utilizing Methylation-Specific PCR and gel electrophoresis, we have found that the p15 gene is hypermethylated in Oral Squamous Cell Carcinoma patients. The hypermethylation status of the p15 gene had been previously found in the South Indian population but there is no reported data that demonstrates its hypermethylation status among the North Indian population. The results from this study would be very helpful in shedding light on the potential of the p15 gene as a diagnostic Biomarker. This research contributes to the understanding of epigenetic modifications in Oral Squamous Cell Carcinoma progression in the North Indian population.

Keywords: Promoter hypermethylation, Tumor suppressor gene, Oral Squamous Cell Carcinoma, Epigenetic modification.

https://doi.org/10.33887/rjpbcs/2024.15.3.13

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cancer, a condition in which abnormal cells develop unregulated and are responsible for tissue destruction. OSCC is the most common oral malignancy with an average yearly rate of over fifty thousand cases, making it one of the top oral cancers. Over 90 percent of cases of oral cancer have been determined due to OSCC alone, which also has the greatest rate of death worldwide [1, [2]. According to the WHO, South-East Asia and the European area have the highest rates of OSCC infection and death worldwide. The lips, tongue, upper and lower gums, retromolar triangle, buccal mucosa, floor and roof (palate) of the mouth, oropharynx, and salivary glands are the structures in the mouth that OSCC affects [1, 3]. The lower lip, the floor of the mouth, and the lateral edge of the tongue account for 40% of OSCC occurrences, accordingly [3]. OSCC arises from genetic, epigenetic, and environmental factors, with chronic inflammation and alterations in oncogenes and tumor suppressor genes driving its development. In the GLOBOCAN 2020 report, it was documented that there were 377,713 newly diagnosed cases of oral cancer worldwide each year, resulting in 177,757 deaths annually. Specifically in India, there were 135,929 new cases and 75,290 deaths annually. Oral cancer accounts for 50–70% of all deaths related to cancer in India and is particularly prevalent in Asian countries [4]. The combined use of tobacco and alcohol can lead to a heightened risk of cancer through synergistic effects. Tobacco smoke contains carcinogens, including nicotine, arsenic, and methoxymethylfurfural. Alcohol can activate procarcinogens and facilitate the entry of harmful carcinogens into body cells. As tumors grow, pain may develop, necessitating medical intervention [5].

Conrad Waddington used the term "epigenetics" in the early 1940s to describe how genes react to external factors to form phenotypes and why phenotypic differences could not be purely caused by genetic changes [6]. Epigenetic modifications play crucial roles in regulating the cell cycle, promoting differentiation, and protecting genes from mutations [7]. A phenotype is heritably passed by either mitosis or meiosis, and epigenetic study especially focuses on giving more details about all mechanisms behind its onset, repair, and heredity [8]. Modifications in epigenetics are any genetic variation in gene expression without related variation in DNA base pairs [9]. The epigenetic modification, first observed in cancer cells was DNA methylation which, greatly influences chromatin structure, and gene expression and facilitates steady gene silencing [10]. Gene silencing by promoter hypermethylation plays a huge role in initiating cancer and its progression. During the methylation of DNA, there is a covalent modification of cysteine residues inside the CpG island. DNA transcription is disrupted due to the high methylation rate in the promoter region of the CPG island by altering the binding process of the histone complex [2].

p15 gene, also known as CDKN2B, situated on chromosome 9p21, encodes for the p15 protein, also called $p15^{INK4B}$. p15 plays a pivotal role in regulating the cell cycle by inhibiting cyclin-dependent kinases (CDKs). This inhibition mechanism is crucial for preventing uncontrolled cell proliferation. Moreover, studies reveal the implications of p15 dysregulation in various diseases.

MATERIAL AND METHODOLOGY

Sample Collection: In this study, blood samples were obtained from 20 patients diagnosed with oral squamous cell carcinoma after obtaining informed consent and ethical clearance which were taken from Dharamshila Cancer Hospital & Research Centre, New Delhi. Furthermore, a set of blood samples was obtained from 20 individuals. The patient group exclusively comprised individuals diagnosed with oral squamous cell carcinoma (OSCC), while those without any history of cancer diagnosis were designated as controls.

DNA Extraction: DNA extraction was performed on blood sample. The blood samples underwent lysis, continuation with proteinase K, after which DNA purification was carried out by utilizing phenol-chloroform extraction methodology followed by ethanol precipitation. Subsequently, the isolated sample was resuspended in nuclease-free water and stored at 4°C.

Sodium Bisulfite Modification: This was done by using a Methylation Kit which did not change methylated cytosines and instead changed unmethylated cytosines to uracil.

MS-PCR: The research employed MS-PCR to check the methylation status of the p15 gene in DNA that had been converted using bisulfite modification. The primers that were specifically designed for methylated and unmethylated regions of the p15 gene were used, as shown in **Table 1**. MS-PCR was conducted to examine the hypermethylation status of the p15 gene promoter in individuals diagnosed with OSCC. Gel

electrophoresis was then performed on a 2% agarose gel, with a 50 V voltage current supply to visualize the PCR-amplified fragments of the p15 gene. The PCR procedure began with an initial denaturation for 12 minutes at 95°C, then denaturation for 45 sec at 95°C for 35 cycles, annealing for 45 seconds, and extension at 72°C for sixty seconds. Following a final extension step for 10 min at 72°C.

Table 1: Sequence of the Primers for *p15* Gene

Gene	Sequence (5′–3′)	Annealing temperature	Base pair	Reference
p15 M	F: CGTACAATAACCGAACGACCGA	60°c	94	[15]
	R: GCGTTCGTATTTTGCGGTT			
p15 U	F: CCATACAATAACCAAACAACCAA	60°c	94	
	R: TGTGATGTGTTTGTATTTTGTGGTT			

Abbreviations: M- Methylated, U- Unmethylated, F- Forward, R- Reverse.

RESULTS

DNA isolation

The integrity of the DNA extracted from blood samples were assessed by subjecting it to electrophoresis on a 0.8% agarose gel as shown in **Figure 1**. The resulting bands were visualized using a gel documentation unit.

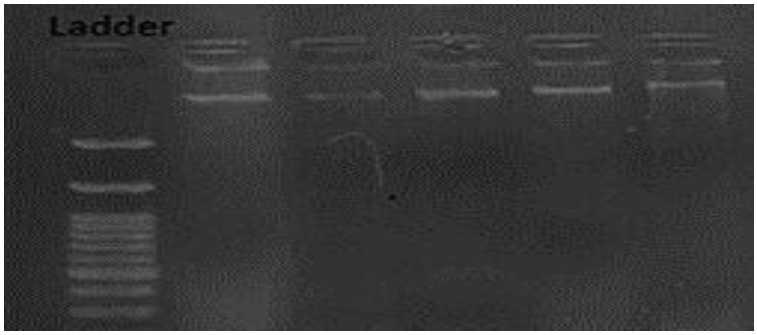

Figure 1: DNA isolated from blood samples of OSCC Patients (Lane No- 1 Ladder, Lane No- 2-6, DNA of OSCC patients)

MSP Result of p15 gene

The result of MS PCR was obtained to evaluate the hypermethylation status of MSP. It was performed to assess the promoter hypermethylation status of the p15 gene in patients with Oral Squamous Cell Carcinoma (OSCC) within the North Indian population. In some samples both Unmethylated and Methylated bands were obtained, the reason could be mixed populations of normal and cancerous cells as shown in **Figure 2**.

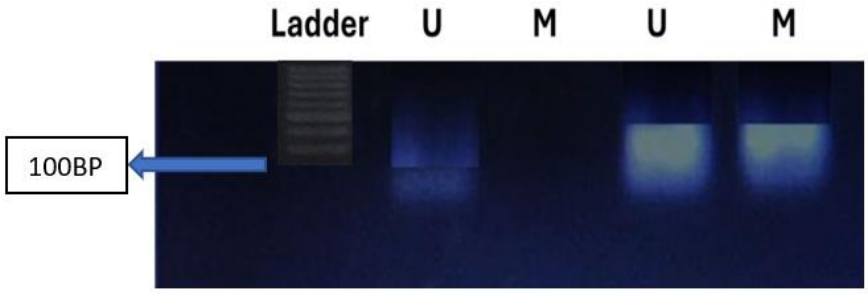

Figure 2: Lane No- 1 represents molecular marker; Lane No-2,4 represents Unmethylated bands; Lane No- 3,5 represents Methylated bands.

DISCUSSION

It is important to note that epigenetic changes can vary across populations, largely influenced by environmental and dietary factors. Therefore, understanding these variations is essential for elucidating OSCC etiology and developing targeted therapeutic interventions. p15 gene, also known as CDKN2B, situated on chromosome 9p21, encodes for the p15 protein and is a tumor suppressor gene. The previous study shows that the p15 gene is hypermethylated in lung cancer, cervical cancer, ovarian cancer, and cancer of breast [7, 11-13] and in OSCC (Southern Population) of India, the population of Iran and the population of Spain [14-16]. This study marks the first report of the p15 gene among oral squamous cell carcinoma (OSCC) patients within the North Indian demographic. This research needs to be verified with a broad sample size to calculate the level of significance of Promoter Hypermethylation of this gene in OSCC among the population of North India. This may lead to the development of p15 Promoter Hypermethylation as a diagnostic biomarker against OSCC.

CONCLUSION

These findings imply that the p15 gene could serve as a promising diagnostic biomarker. This study represents the first documentation of p15 gene hypermethylation in OSCC patients within the North Indian population.

ACKNOWLEDGMENT

The authors acknowledge the help provided by the Department of Biotechnology, Sharda School of Engineering and Technology, Sharda University, Greater Noida, India.

Abbreviations

OSCC- Oral Squamous Cell Carcinoma
CDKs- Cyclin-Dependent Kinases
PCR- Polymerase Chain Reaction
MS- PCR- Methylation Specific Polymerase Chain Reaction

REFERENCES

[1] Maleki D, Ghojazadeh M, Mahmoudi SS, Mahmoudi SM, Pournaghi-Azar F, Torab A, Piri R, Azami-Aghdash S, Naghavi-Behzad M. Epidemiology of oral cancer in Iran: a systematic review. Asian Pacific Journal Of Cancer Prevention 2015;16(13):5427-32.

[2] Liu D, Zhao X, Zeng X, Dan H, Chen Q. Non-invasive techniques for detection and diagnosis of oral potentially malignant disorders. The Tohoku Journal Of Experimental Medicine 2016;238(2):165-77.

[3] Pałasz P, Adamski Ł, Górska-Chrząstek M, Starzyńska A, Studniarek M. Contemporary diagnostic imaging of oral squamous cell carcinoma–a review of literature. Polish Journal Of Radiology 2017;82:193.

[4] Kumar M, Jha AK. Exploring the potential of dietary factors and plant extracts as chemopreventive agents in oral squamous cell carcinoma treatment. Frontiers in Oral Health 2023;4.

[5] D'souza S, Addepalli V. Preventive measures in oral cancer: An overview. Biomedicine & Pharmacotherapy 2018; 107:72-80.

[6] Tronick E, Hunter RG. Waddington, dynamic systems, and epigenetics. Frontiers in Behavioral Neuroscience 2016;10:107.

[7] Jha AK, Nikbakht M, Jain V, Capalash N, Kaur J. p16INK4a and p15INK4b gene promoter methylation in cervical cancer patients. Oncology Letters 2012;3(6):1331-5.

[8] Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. Genes & development 2009;23(7):781-3.

[9] Mascolo M, Siano M, Ilardi G, Russo D, Merolla F, De Rosa G, Staibano S. Epigenetic disregulation in oral cancer. International Journal Of Molecular Sciences 2012;13(2):2331-53.

[10] Goel H, Mathur R, Syeda S, Shrivastava A, Jha AK. Promoter hypermethylation of LATS1 gene in oral squamous cell carcinoma (OSCC) among North Indian population. Asian Pacific Journal of Cancer Prevention: APJCP 2021;22(3):977.

[11] Kurakawa E, Shimamoto T, Utsumi K, Hirano T, Kato H, Ohyashiki K. Hypermethylation of p16INK4a and p15INK4b genes in non-small cell lung cancer. International Journal Of Oncology 2001;19(2):277-81.
[12] Moselhy SS, Kumosani TA, Kamal IH, Jalal JA, Jabaar HS, Dalol A. Hypermethylation of P15, P16, and E-cadherin genes in ovarian cancer. Toxicology And Industrial Health 2015;31(10):924-30.
[13] Qi M, Xiong X. Promoter hypermethylation of RARβ2, DAPK, hMLH1, p14, and p15 is associated with progression of breast cancer: A PRISMA-compliant meta-analysis. Medicine 2018;97(51):e13666.
[14] Viswanathan M, Tsuchida N, Shanmugam G. Promoter hypermethylation profile of tumor-associated genes p16, p15, hMLH1, MGMT and E-cadherin in oral squamous cell carcinoma. International Journal of Cancer 2003;105(1):41-6.
[15] Kordi-Tamandani DM, Ladies MA, Hashemi M, Moazeni-Roodi AK, Krishna S, Torkamanzehi A. Analysis of p15 ink4b and p16 ink4a gene methylation in patients with oral squamous cell carcinoma. Biochemical Genetics 2012;50:448-53.
[16] Bonar-Alvarez P, Perez-Sayans M, Padín-Iruegas E, Suarez-Penaranda JM, Oliveira-Alves M, Blanco Carrion A, García-Garcia A. State of methylation and epigenome studies in some types of head and neck cancer. Biomedical Research (India) 2018:2944-55.