

Access this article online

Quick Response Code:



Website:

www.carcinogenesis.com

DOI:

10.4103/jcar.JCar_5_16

Role of matrix metalloproteinase 13 gene expression in the evaluation of radiation response in oral squamous cell carcinoma

Shankar Sharan Singh, Madan Lal Brahma Bhatt, Vandana Singh Kushwaha, Anshuman Singh¹, Rajendra Kumar, Rajeev Gupta, Devendra Parmar¹

Abstract:

BACKGROUND: Matrix Metalloproteinase 13 (MMP13) is a member of collagenase family and it is involved in the degradation of extracellular matrix and basement membrane protein. It is thought to be associated with tumor invasion and metastasis. Elevated MMP13 expression has been found in carcinoma of the breast, urinary bladder, head and neck and others. It is observed that MMP13 gene is also correlated with radiation response in OSCC (Oral squamous cell carcinoma) cell line based study. The present study correlates the MMP13 expressions with clinicopathological parameters and radiation response in OSCC patients. **MATERIALS AND METHODS:** The MMP13 mRNA levels were determined by employing qRT-PCR (real-time quantitative reverse transcriptase-polymerase chain reaction). **RESULTS:** We observed high expression of MMP13 mRNA in OSCC patients when compared with matched controls. Statistically significant up regulation of MMP13 mRNA expression was found in tobacco chewers, advanced T-stage ($p < 0.001$) and lymph node metastasis ($p < 0.01$). MMP13 mRNA levels were also elevated in non responders as compared to responders to radiation treatment. **CONCLUSIONS:** To the best of our knowledge, this is the first report that indicates role of MMP13 in radiation response in OSCC patients and could be used as potential bio-marker for radiotherapy treatment in OSCC patients.

Keywords:

Matrix Metalloproteinase 13, oral squamous cell carcinoma, radiation response

Introduction

Oral squamous cell carcinoma (OSCC) is the 12th most common cancer occurring worldwide. It is more common in males and accounts for more than 30% of all cancers in India.^[1] Radiation therapy with or without chemotherapy is used as a primary or adjuvant treatment in advanced OSCC.^[2] Despite advances in treatment, only 50% of the patients who underwent radiotherapy have sustained response.^[3] It may be due to variability in tumor genetics and biology. Currently, there is no available marker which can

reliably predict the radiotherapy response in such patients.

Matrix Metalloproteinase 13 (MMP13) gene encodes a protein, involved in the breakdown of extracellular matrix in normal physiological processes. Previous studies demonstrated that MMP13 is highly overexpressed in pathological conditions, such as arthritis and human carcinomas.^[4] MMP13 is the essential protein for tumor invasion and metastatic spread and its expression may help predict the radiotherapy response and prognosis in OSCC patients. Our objective was to examine the expression of MMP13 in relation to clinicopathological

Department of
Radiotherapy,
King George's Medical
University, ¹Division of
Developmental Toxicology,
CSIR Indian Institute of
Toxicology Research,
Lucknow, Uttar Pradesh,
India

Address for correspondence:

Dr. Madan Lal Brahma
Bhatt,
Department of
Radiotherapy,
King George's
Medical University,
Lucknow - 226 003,
Uttar Pradesh, India.
E-mail: drmlbhatt@yahoo.
com

Received : 09 July, 2016
Accepted : 11 April, 2017
Published: 19 June, 2017

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Singh SS, Bhatt ML, Kushwaha VS, Singh A, Kumar R, Gupta R, *et al.* Role of matrix metalloproteinase 13 gene expression in the evaluation of radiation response in oral squamous cell carcinoma. J Carcinog 2017;16:2.

features of OSCC patients and with their radiotherapy response.

Materials and Methods

Between 2010 and 2013, 160 histopathologically confirmed tissue samples from OSCC patients (as recommended for the radiotherapy treatment) as well as eighty matched controls were obtained from King George's Medical University, Lucknow. The study was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants before the recruitment in the study. The patients had not received prior radiotherapy, chemotherapy, or neoadjuvant therapies for their cancer or any other disease in the past. The characteristics of the OSCC patients including age, clinical stage (TNM classification defined by the American Joint Committee on Cancer (AJCC) 2010, seventh edition)^[5] and habits (Tobacco, alcohol, smoking) were assessed by Radiation Oncologist.

Analysis of mRNA expression by real-time quantitative reverse-transcriptase polymerase chain reaction

The tissue specimens of OSCC patients and controls were collected in TRIzol Reagent (Ambion, Life Technology) and immediately homogenized with a T25 basic homogenizer (IKA Labortechnik, Staufen, Germany). Total RNA was isolated according to TRIzol Reagent protocol and stored at -80°C for further use. The total RNA of each sample was reverse transcribed to cDNA using verso cDNA synthesis kit (Thermo Scientific, EU, Lithuania) according to the manufacturer's protocol. Real-time quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) analysis was performed according to the manufacturer protocol (Takara SYBR fast qPCR kit, Takara Biosystems). The qRT-PCR reactions were performed using Applied Biosystems 7900HT real-time PCR system, and reaction was carried out in triplicate. Beta-actin was taken as an endogenous control and the cDNA from matched control tissue (RQ = 1) was utilized for the normalization of test samples. The primer sets specific for MMP13 (Forward: 5'-TTCCACAGTGCCTATTGATAC-3', Reverse: 5'-ATCAACAGTGTCTCTGAGCAGCA-3') and for beta-actin (Forward: 5'-GCACA GAGCCTCGCCTT-3', Reverse: 5'-GTTGTGACGACGAGCG-3') were used. An initial incubation of enzyme denaturation at 95°C for 10 min followed by 45 rounds of amplification at 95°C (10 s) for denaturation, 58°C – 65°C (10 s) for annealing, and 72°C (30 s) for extension for performing qRT-PCR. Relative change in mRNA levels between tumor and matched normal control tissue was calculated using $2^{-\Delta\Delta\text{Ct}}$ method.

Proposed methodology

All patients were subjected to radiotherapy using telecobalt radiotherapy machine. A dose of 70 Gy of radiation was delivered in 7 weeks with 2 Gy fraction size, 5 days a week (46 Gy to primary and whole neck followed by 24 Gy after sparing the cord) with concurrent cisplatin $35\text{ mg/m}^2/\text{week}$. Radiation response was evaluated 1 month after the completion of radiotherapy treatment using WHO criteria.^[6]

Statistical analysis

One-way ANNOVA using Turkey's multiple comparison test was used to compare the mRNA expression levels between different groups in both cases and controls. Comparisons were made between categorical groups by Chi-square test. A two-tailed $P < 0.05$ was considered statistically significant.

Results

Matrix metalloproteinase 13 mRNA expression and basic characteristics of oral squamous cell carcinoma patients

The demographic and clinicopathological parameters of OSCC patients are summarized in Table 1. The median age of patients was 48 years. Among 160 patients, majority were males (76.2%), with majority having lesion at buccal mucosa (55%). Out of 122 males, tobacco chewing habit was present in 80 (65.6%) patients, 68 (55.7%) patients were smokers, and 63 (51.6%) patients had a history of alcohol consumption, while in females, 22 (57.9%) were tobacco chewers, 16 (42.1%) were smokers, and 17 (44.7%) patients had alcohol consumption habit. Most of the patients had tumor size.

The qRT-PCR analysis revealed highly significant ($P < 0.001$) induction in the mRNA expression of MMP13 in OSCC patients as compared to matched controls [Figure 1].

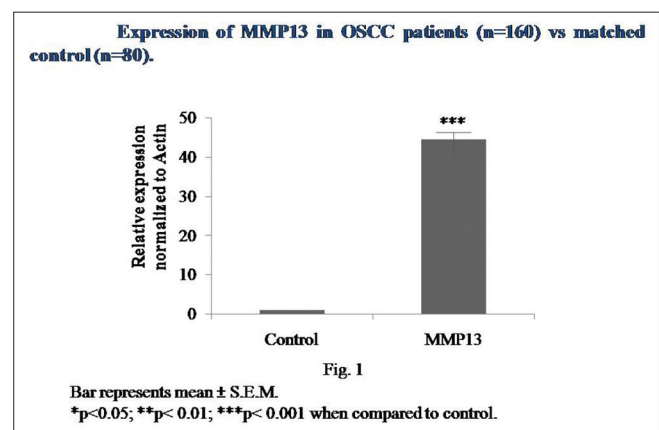


Figure 1: MMP13 mRNA expression in OSCC patients and controls

Expression of matrix metalloproteinase 13 mRNA in clinicopathological characteristics

Overexpression of MMP13 mRNA was significantly correlated with chewing habit ($P < 0.01$), advanced tumor size (T3 and T4, $P < 0.001$), advanced stage and lymph node metastasis ($P < 0.01$) in OSCC patients [Table 1].

Association of matrix metalloproteinase 13 mRNA expression with the radiation response

Out of 160 patients, 92 (57.5%) patients responded to radiotherapy (complete response and partial response) and 68 (42.5%) were nonresponders (no response and progressive disease) [Table 2]. The expression of MMP13 mRNA was found to be significantly higher ($P < 0.001$) in nonresponders as compared to responders.

qRT-PCR analysis further revealed a highly significant ($P < 0.001$) expression of MMP13 mRNA in patients who were tobacco chewers, along with smoking and alcohol consumption habits, as compared to patients

who were tobacco chewers or alcoholic in both nonresponders and responders [Figure 2]. The MMP13 mRNA expression was highly significant in males who were tobacco chewers, alcoholics, and smokers

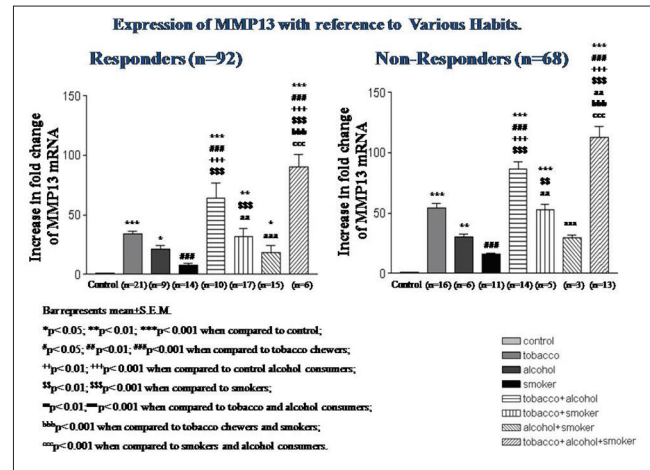


Figure 2: Matrix metalloproteinase 13 mRNA expression according to the consumption of tobacco (chewer and smoker) and alcohol

Table 1: Correlation of matrix metalloproteinase 13 gene expression with the clinicopathological characteristics

Variables	Category	Number of patients (%)	MMP13 mRNA expression		P
			Low expression, n (%)	High expression, n (%)	
Total		160	57 (35.6)	103 (64.4)	
Age (years)	<45	59 (46.9)	22 (37.3)	37 (62.7)	0.8692
	≥ 45	101 (53.1)	35 (34.7)	66 (65.3)	
Gender	Female	38 (23.8)	17 (44.7)	21 (55.2)	0.2505
	Male	122 (76.3)	40 (32.8)	82 (67.2)	
Smoking	No	76 (47.5)	24 (31.6)	52 (68.4)	0.3946
	Yes	84 (52.5)	33 (39.3)	51 (60.7)	
Drinking	No	84 (55)	36 (42.9)	48 (57.1)	0.0653
	Yes	76 (45)	21 (27.6)	55 (72.4)	
Tobacco chewing	No	58 (36.3)	27 (46.6)	31 (53.4)	0.045
	Yes	102 (63.8)	30 (29.4)	72 (70.6)	
Primary site	Buccal mucosa	88 (55)	27 (30.7)	61 (69.3)	0.2014
	Others*	72 (45)	30 (41.7)	42 (58.3)	
SCC differentiation	Well	73 (45.6)	24 (32.9)	49 (67.1)	0.6176
	Poor and Moderate	87 (54.4)	33 (37.9)	54 (62.1)	
Tumor size	T1–T2	45 (28.1)	24 (53.3)	21 (46.7)	0.0061
	T3–T4	115 (71.9)	33 (28.7)	82 (71.3)	
Lymph node metastasis	Negative	64 (40)	31 (48.4)	33 (51.6)	0.0095
	Positive	96 (60)	26 (27.1)	70 (72.9)	
Stage	Early stage	19 (11.9)	13 (68.4)	6 (26.3)	0.0034
	Advanced stage	141 (88.1)	44 (31.2)	97 (69.5)	

*Others: Tongue, alveolus, floor of mouth, lip, soft palate. Significant P values were highlighted in bold. MMP13: Matrix metalloproteinase 13, SCC: Squamous cell carcinoma

Table 2: Association of matrix metalloproteinase-13 gene expression with the radiation response

Radiotherapy treatment response	Number of patients (n=160), n (%)	MMP13 mRNA expression		P
		Low level of expression, n (%)	High level of expression, n (%)	
Responders	92 (57.5)	45 (48.9)	47 (51.1)	<0.001
Nonresponders	68 (42.5)	12 (17.6)	56 (82.4)	

MMP13: Matrix metalloproteinase 13

in both responders and nonresponders; however, a significant association of MMP13 mRNA was only found in responder female patients who were only tobacco chewers [Tables 3 and 4].

The overexpression of MMP13 mRNA was found to be highly significant in early T-stage (T1, T2) and advanced T-stage (T3, T4) of nonresponders as compared to responders [Figure 3]. Similarly, highly significant increase in MMP13 mRNA expression was also found in both lymph node positive and negative nonresponders as compared to responders [Figure 4].

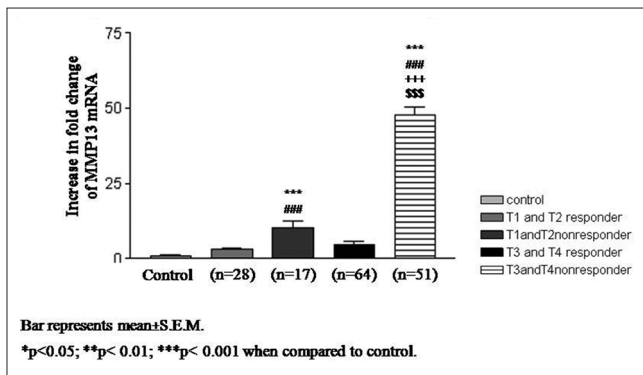


Figure 3: Matrix metalloproteinase 13 mRNA expression according to different T-stages of oral squamous cell carcinoma

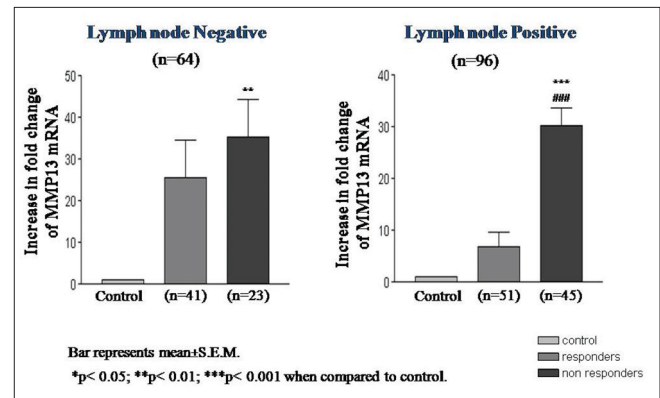


Figure 4: Matrix metalloproteinase 13 mRNA expression according to lymph nodes status of oral squamous cell carcinoma

Table 3: Correlation of matrix metalloproteinase 13 mRNA expression radiotherapy treatment responders (males and females)

Gender	Number of cases, n (%)	Radiotherapy treatment response Responders, n (%)	MMP13 mRNA expression		P
			Low level of expression, n (%)	High level of expression, n (%)	
Male					
Total	122	69	31	38	
Tobacco chewing					0.003
Yes	80 (65.6)	44 (63.8)	12 (38.7)	32 (84.2)	
No	42 (34.4)	25 (36.2)	19 (61.3)	6 (15.8)	
Smoking					0.0312
Yes	68 (55.7)	38 (55.1)	22 (71)	16 (42.1)	
No	54 (44.3)	31 (44.9)	9 (29)	22 (57.9)	
Drinking					0.0052
Yes	63 (51.6)	35 (50.7)	22 (71)	13 (34.2)	
No	59 (48.4)	34 (49.3)	9 (29)	25 (65.8)	
Female					
Total	38	23	14	9	
Tobacco chewing					0.025
Yes	22 (57.9)	10 (43.5)	3 (21.4)	7 (77.8)	
No	16 (42.1)	5 (56.5)	11 (78.6)	2 (22.2)	
Smoking					0.2623
Yes	16 (42.1)	6 (26.1)	2 (14.3)	4 (44.4)	
No	22 (57.9)	17 (73.9)	12 (85.7)	5 (55.6)	
Drinking					0.0604
Yes	17 (44.7)	11 (47.8)	4 (28.6)	7 (77.8)	
No	21 (55.3)	12 (52.2)	10 (71.4)	2 (22.2)	

MMP13: Matrix metalloproteinase 13

Discussion

Our hypothesis was that MMP13 might be overexpressed in OSCC and its expression could be correlated with clinicopathological parameters and radiation response. The significant expression of MMP13 was found in previous studies in different types of human cancer such as breast cancer, squamous cell carcinomas of the head and neck and vulva, malignant melanomas, chondrosarcomas, and urinary bladder cancer.^[7-11] Several studies demonstrated that MMP13 was overexpressed in oral cancer.^[12,13] Similarly, this

Table 4: Correlation of matrix metalloproteinase 13 mRNA expression radiotherapy treatment nonresponders (males and females)

Gender	Number of cases, n (%)	Radiotherapy treatment response Nonresponders, n (%)	MMP13 mRNA expression		P
			Low level of expression, n (%)	High level of expression, n (%)	
Male					
Total	122	53	7	46	
Tobacco chewing					0.0023
Yes	80 (65.6)	42 (79.2)	2 (28.6)	40 (87)	
No	42 (34.4)	11 (20.8)	5 (71.4)	6 (13)	
Smoking					0.043
Yes	68 (55.7)	30 (56.6)	1 (14.3)	29 (63)	
No	54 (44.3)	14 (43.4)	6 (85.7)	8 (37)	
Drinking					0.0233
Yes	63 (51.6)	38 (71.7)	2 (28.6)	36 (78.3)	
No	59 (48.4)	15 (28.3)	5 (71.4)	10 (21.7)	
Female					
Total	38	15	5	10	
Tobacco chewing					0.4936
Yes	22 (57.9)	12 (80)	3 (60)	9 (90)	
No	16 (42.1)	3 (20)	2 (40)	1 (10)	
Smoking					0.8465
Yes	16 (42.1)	10 (66.7)	4 (80)	6 (60)	
No	22 (57.9)	5 (33.3)	1 (20)	4 (40)	
Drinking					0.5762
Yes	17 (44.7)	6 (40)	3 (60)	3 (30)	
No	21 (55.3)	9 (60)	2 (40)	7 (70)	

MMP13: Matrix metalloproteinase 13

study also found the significant increase in the mRNA expression of MMP13 in OSCC patients as compared to matched controls.

Recently, Vincent-Chong *et al.* demonstrated that overexpression at protein level and mRNA level of MMP3 was found in OSCC patients but only at protein level of expression showed significant association with lymph node metastasis and tumor staging.^[12] In the current study, we observed the significant overexpression of mRNA of MMP13 in tobacco chewers, tumor size, advanced stage, and lymph node metastasis. This observation reflects the mRNA level activity of MMP13 which may be involved in the tumor progression of OSCC.

We also found that the significant increase in mRNA expression of MMP13 was found to be more in OSCC patients, who were tobacco chewers, smokers, and alcohol consumers when compared with matched controls.

Several genomic-based studies demonstrated that significantly higher expression of MMP13 was found in advanced stage of OSCC patients.^[12,13] The present findings are in accordance with the previous study demonstrating increase in the expression MMP13 gene in advanced stage.

Currently, radiation therapy has played an imperative constituent in controlling tumor growth and providing cure for patients with OSCC. However, radiotherapy is sometimes ineffective against cancer cells which show resistance to radiation.^[14,15] A cell line-based study demonstrated that this gene was reported to be highly expressed in radio-resistant cells when compared to radiosensitive cells in OSCC.^[14] Our data demonstrated increase in the expression of MMP13 to be associated with radio-resistant in OSCC patients.

Our results also revealed that MMP13 gene was also significantly expressed in early as well as advanced stage of OSCC patients who were nonresponders after radiotherapy treatment. The overexpression of MMP13 mRNA levels in nonresponders may further provide support that MMP13 is involved in the invasion and progression of OSCC.

No previous studies have evaluated association between clinicopathological parameters and radiotherapy treatment response in OSCC patients. This gene may help in finding the pattern of radio-resistance in OSCC cases on the basis of substance abuse, lymph node metastasis, and stage of the tumor.

Conclusion

We observed a significant increase in the mRNA expression of MMP13 in OSCC patients as compared to matched controls. Overexpression of MMP13 gene was found to be significantly higher in advanced stage when compared to early stage. The patients who had lower mRNA expression responded better to radiation treatment as compared to a patient who had higher mRNA expression. These results may lead to the consideration that altered level of this gene may be helpful in predicting radiotherapy treatment response in OSCC patients. These results advance our knowledge involving this gene in the resistance to the response of radiation therapy and could be used as potential marker for the radiotherapy treatment. However, larger studies are required to understand the role of MMP13 expression, its predictive significance for radiotherapy outcome in OSCC patients and incorporation of this in the routine clinical practice.

Financial support and sponsorship

This study was financially supported by the Indian Council Medical Research, New Delhi, India.

Conflicts of interest

There are no conflicts of interest.

References

- Misra S, Chaturvedi A, Misra NC. Management of Gingivobuccal Complex Cancer. Vol. 25. London: Royal Society of Medicine Press; 2002. p. 71-86.
- Gupta AK, Cerniglia GJ, Mick R, Ahmed MS, Bakanauskas VJ, Muschel RJ, *et al.* Radiation sensitization of human cancer cells *in vivo* by inhibiting the activity of PI3K using LY294002. *Int J Radiat Oncol Biol Phys* 2003;56:846-53.
- Feller L, Lemmer J. Oral squamous cell carcinoma: Epidemiology, clinical presentation and treatment. *J Cancer Ther* 2012;3:263-8.
- Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, *et al.* Matrix metalloproteinases: A review. *Crit Rev Oral Biol Med* 1993;4:197-250.
- Edge SB, Compton CC. The American Joint Committee on Cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010;17:1471-4.
- WHO. Handbook for Reporting Results of Cancer Treatment. Geneva, Switzerland: World Health Organization; 1979. p. 1-45.
- Freije JM, Díez-Itza I, Balbín M, Sánchez LM, Blasco R, Tolivia J, *et al.* Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. *J Biol Chem* 1994;269:16766-73.
- Johansson N, Airola K, Grénman R, Kariniemi AL, Saarialho-Kere U, Kähäri VM. Expression of collagenase-3 (matrix metalloproteinase-13) in squamous cell carcinomas of the head and neck. *Am J Pathol* 1997;151:499-508.
- Airola K, Karonen T, Vaalamo M, Lehti K, Lohi J, Kariniemi AL, *et al.* Expression of collagenases-1 and -3 and their inhibitors TIMP-1 and -3 correlates with the level of invasion in malignant melanomas. *Br J Cancer* 1999;80:733-43.
- Uría JA, Balbín M, López JM, Alvarez J, Vizoso F, Takigawa M, *et al.* Collagenase-3 (MMP-13) expression in chondrosarcoma cells and its regulation by basic fibroblast growth factor. *Am J Pathol* 1998;153:91-101.
- Boström PJ, Ravanti L, Reunanen N, Aaltonen V, Söderström KO, Kähäri VM, *et al.* Expression of collagenase-3 (matrix metalloproteinase-13) in transitional-cell carcinoma of the urinary bladder. *Int J Cancer* 2000;88:417-23.
- Vincent-Chong VK, Salahshourifar I, Karen-Ng LP, Siow MY, Kallarakkal TG, Ramanathan A, *et al.* Overexpression of MMP13 is associated with clinical outcomes and poor prognosis in oral squamous cell carcinoma. *Scientific World Journal* 2014;2014:897523.
- Chiang WC, Wong YK, Lin SC, Chang KW, Liu CJ. Increase of MMP-13 expression in multi-stage oral carcinogenesis and epigallocatechin-3-gallate suppress MMP-13 expression. *Oral Dis* 2006;12:27-33.
- Ishigami T, Uzawa K, Higo M, Nomura H, Saito K, Kato Y, *et al.* Genes and molecular pathways related to radioresistance of oral squamous cell carcinoma cells. *Int J Cancer* 2007;120:2262-70.
- Terakado N, Shintani S, Yano J, Chunnan L, Mihara M, Nakashiro K, *et al.* Overexpression of cyclooxygenase-2 is associated with radioresistance in oral squamous cell carcinoma. *Oral Oncol* 2004;40:383-9.

Authors Profile

Shankar Sharan Singh: Department of Radiotherapy, King George's Medical University, Lucknow, Uttar Pradesh, India

Madan Lal Brahma Bhatt: Department of Radiotherapy, King George's Medical University, Lucknow, Uttar Pradesh, India

Vandana Singh Kushwaha: Department of Radiotherapy, King George's Medical University, Lucknow, Uttar Pradesh, India

Anshuman Singh: Division of Developmental Toxicology, CSIR Indian Institute of Toxicology Research, Lucknow, Uttar Pradesh, India


Rajendra Kumar: Department of Radiotherapy, King George's Medical University, Lucknow, Uttar Pradesh, India

Uttar Pradesh, India

Rajeev Gupta: Department of Radiotherapy, King George's Medical University, Lucknow, Uttar Pradesh, India

Devendra Parmar: Division of Developmental Toxicology, CSIR Indian Institute of Toxicology Research, Lucknow, Uttar Pradesh, India

Journal of Carcinogenesis is published for Carcinogenesis Press by Medknow Publications and Media Pvt. Ltd.

 Manuscripts submitted to the journal are peer reviewed and published immediately upon acceptance, cited in PubMed and archived on PubMed Central. Your research papers will be available free of charge to the entire biomedical community. Submit your next manuscript to Journal of Carcinogenesis.

www.journalonweb.com/jcar/