Original Research

Deciphering the role of TLR3 polymorphisms in oral squamous cell carcinoma pathogenesis: A case-control study

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Abstract

Background: Oral squamous cell carcinoma (OSCC) poses a significant global health burden, particularly prevalent in regions like India. Despite advancements in diagnostics, early detection of OSCC remains challenging, necessitating novel diagnostic modalities. Toll-like receptors (TLRs) and their polymorphisms have emerged as potential contributors to OSCC pathogenesis.

Methods: This retrospective case-control study examined 120 individuals, including 60 OSCC cases and 60 healthy controls. Genotyping of TLR3 single-nucleotide polymorphisms (SNPs) rs3775290 and rs3775291 was conducted using TaqMan allelic discrimination real-time polymerase chain reaction. Functional consequence analysis and TLR3 expression profiling were performed to elucidate their role in OSCC pathogenesis.

Results: Significant associations were observed between TLR3 SNPs and OSCC susceptibility, particularly at loci rs3775290 and rs3775291. Functional consequence analysis revealed pathogenic mutations in TLR3 genes, potentially affecting protein structure and function. TLR3 overexpression was detected in OSCC lesions, implicating its involvement in disease progression.

Conclusion: TLR3 polymorphisms play a pivotal role in OSCC pathogenesis, offering potential biomarkers for diagnosis and prognosis. Targeting TLR3-mediated pathways may hold promise in personalised OSCC management. Further research is warranted to elucidate the precise mechanisms underlying TLR3-mediated carcinogenesis in OSCC, facilitating the development of tailored therapeutic strategies.

Keywords: OSCC, single-nucleotide polymorphisms, toll-like receptors

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INTRODUCTION

Oral cancer represents a significant global health challenge, particularly prevalent in developing countries like India, where it accounts for over 30% of all malignancies.[1] Annually, India reports approximately 77,000 new cases and 52,000 deaths from oral cancer, contributing substantially to the global burden of the disease.[1,2] Deleterious habits such as smoking, smokeless tobacco use, and alcohol consumption are major causative factors, with synergistic effects noted, particularly with alcohol consumption.[3] Patients with oral cancer face varying prognoses, highly dependent on the disease stage at presentation.[3]

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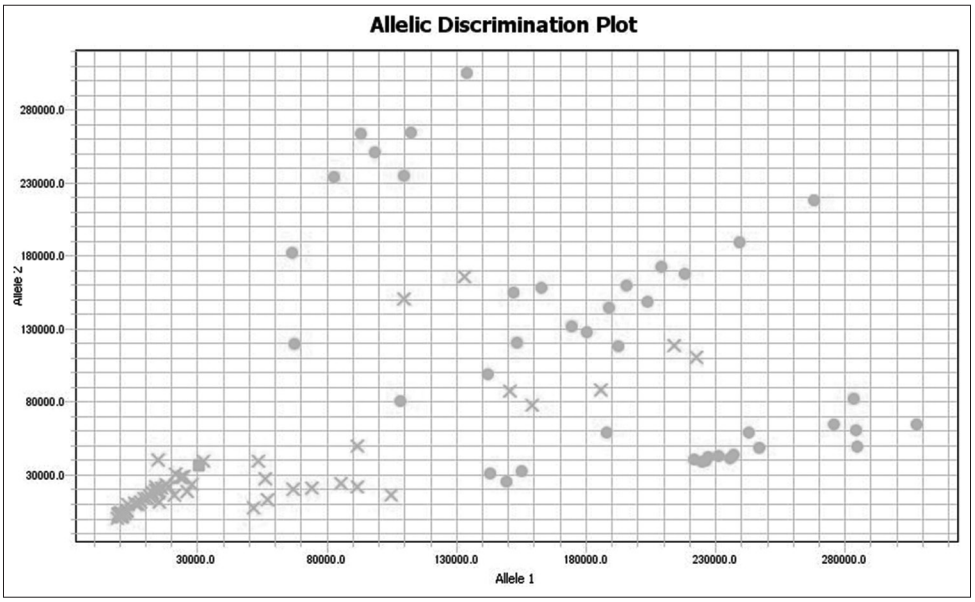
Despite advances in diagnostic techniques, limitations persist, hindering early-stage detection and accurate differentiation of premalignant lesions.[3,4] Consequently, there is an urgent need for novel, rapid, and precise diagnostic modalities for oral carcinoma detection.[3]

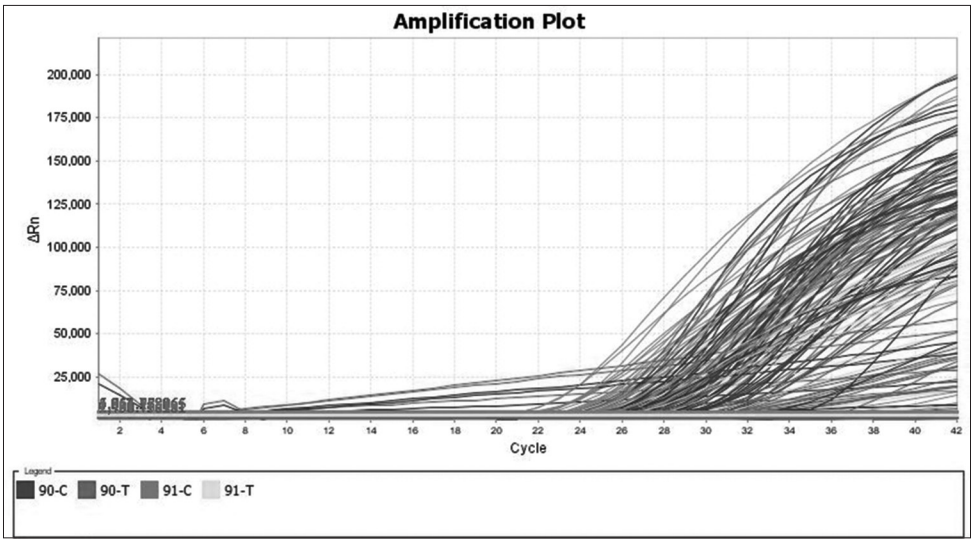
Chronic inflammatory processes and molecular pathways, notably the Toll-like receptors (TLRs) pathway, have been implicated in oral squamous cell carcinoma (OSCC) pathogenesis.[5,6] TLRs play a critical role in innate immunity, recognising pathogen-associated molecular patterns (PAMPs) and activating signaling cascades that contribute to tumour progression.[6] Recent studies have suggested a potential link between TLR3 polymorphisms and cancer risk, particularly at loci rs3775290 and rs377529.[3,6]

Given the ambiguity surrounding the association between TLR3 polymorphisms and OSCC risk, comprehensive research in this area is warranted.[6] This study aims to elucidate the relationship between TLR3 single-nucleotide polymorphisms (SNPs) and OSCC risk, shedding light on the genetic factors contributing to disease susceptibility.[6] By exploring the role of genetic variants in the TLR3 gene polymorphism, particularly at hotspot SNPs rs3775290 and rs3775291, this study seeks to provide valuable insights into the molecular mechanisms underlying oral carcinoma development.[6]

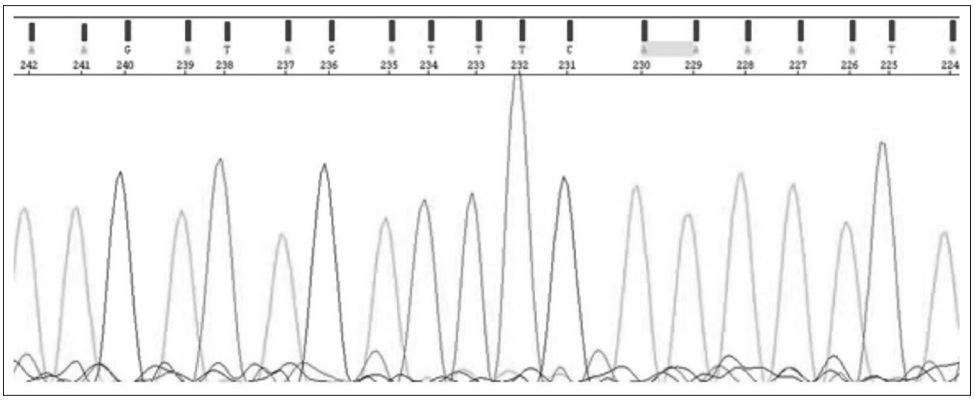
The significance of this research lies in its potential to enhance our understanding of the genetic basis of oral cancer, paving the way for personalised diagnostic



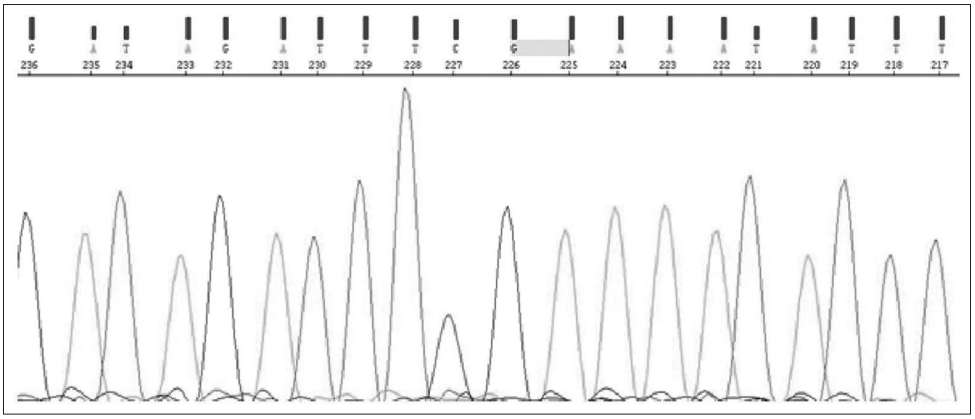
Graph 1: Allelic discrimination Plot with wild and mutant lying in y and x axis while hetro in to centre



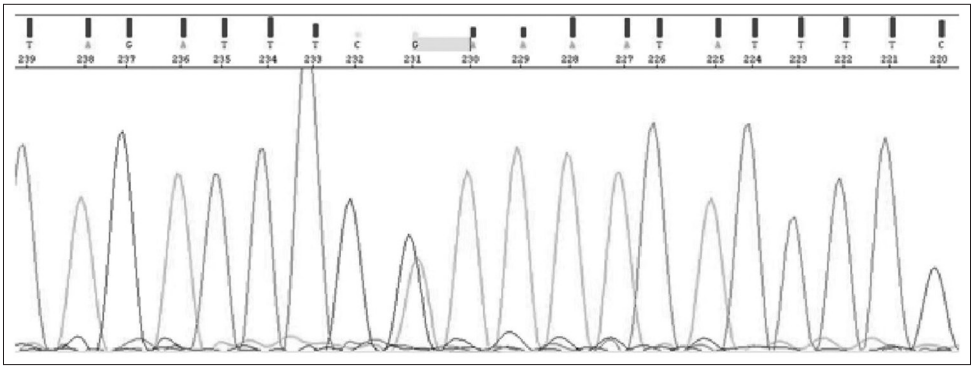
Graph 2: Real Time Graph of allelic discrimination assay



Graph 3: Graph showing wild, mutant and heterozygous condition in rs3775290 - A/G (C/T)



Graph 4: Graph showing wild, mutant and heterozygous condition in rs3775290 AG



Graph 5: Graph showing wild, mutant and heterozygous condition in rs3775290 G

and therapeutic approaches. Moreover, elucidating the role of TLR3 polymorphisms in OSCC risk may have broader implications for cancer prevention and treatment strategies.

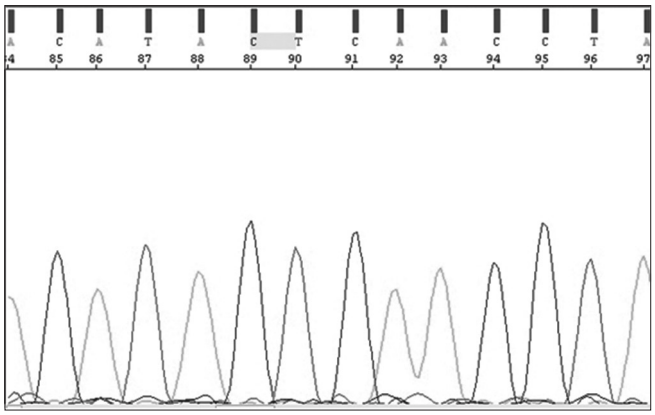
MATERIALS AND METHODS

This retrospective case-control study involved the examination of 120 cases, comprising 60 individuals diagnosed with OSCC and 60 healthy controls, sourced from the Oral and Maxillofacial Pathology department at Sardar Patel Post Graduate Institute of Dental and Medical Sciences (SPPGIDMS), Lucknow. Institutional Ethics Committee approval was obtained prior to commencement.

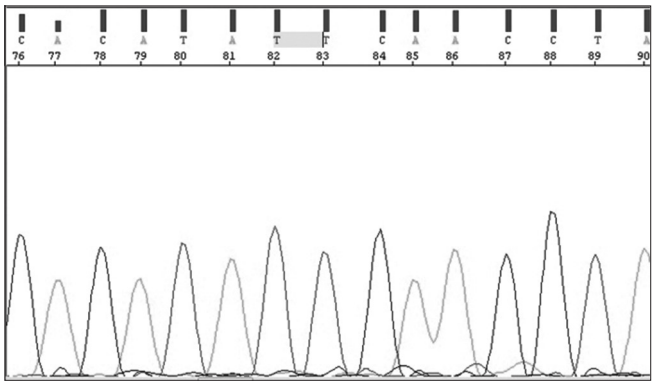
The study population was divided into three groups:

Group 1 consisted of healthy individuals,

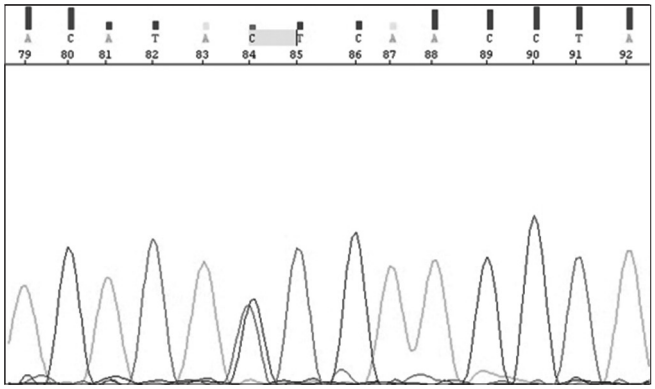
Group 2 included various grades of OSCC [Well Differentiated (WDSCC), Moderately Differentiated (MDSCC), and Poorly Differentiated (PDSCC)], and



Graph 6: Graph showing wild, mutant and heterozygous condition in rs3775291 C



Graph 7: Graph showing wild, mutant and heterozygous condition in rs3775291 T



Graph 8: Graph showing wild, mutant and heterozygous condition in rs3775291 CT

Group 3 focused specifically on Poorly Differentiated OSCC. All laboratory work was conducted at IMaGe Diagnostics and Research Centre, Lucknow.

For DNA purification, the Purelink Genomic DNA kit was utilized. Formalin-fixed, paraffin-embedded tissue lysates underwent centrifugation and ethanol addition to wash buffers, followed by DNA extraction. Elution was performed using PureLink Genomic Elution Buffer, ensuring purification of genomic DNA [Graphs 1 and 2].

Genotyping of SNPs for rs3775290 [Graphs 3-5] and rs3775291[Graphs 6-8] in TLR-3 was conducted using TaqMan allelic discrimination real-time polymerase chain reaction (PCR). Customised primers and probes provided by ThermoFisher Scientific were employed in a PCR mixture comprising SNP Genotyping Assay, PCR Master Mix, and DNA. Amplifications were carried out under specific thermal cycling conditions, and genotyping discrimination was performed using real-time fluorescence detection software.

For rs3775290 (1377 C/T) and rs3775291 (L412F), detailed information including SNP ID, Gene (TLR3), Gene Name (toll-like receptor 3), Chromosome Location, Polymorphism, and Context Sequence was recorded. Sequencing was conducted on 10% of samples for each SNP using an ABI Sanger sequencer to validate results.

A) 1377 C/T (rs3775290): Graph 3-5
- SNP ID: rs3775290
- Gene: TLR3
- Gene Name: toll-like receptor 3
- Chromosome Location: Chr. 4: 186083063-186083063 on Build GRCh38
- Polymorphism: C/T, Transition Substitution
- Context Sequence [VIC/FAM]: AATGGAGAGGTCTAGAAAATATTTT[C/T]GAAATCTATCTTTCCTACAACAAGT

B) L412F (rs3775291): Graph 6-8
- SNP ID: rs3775291
- Gene: TLR3
- Gene Name: toll-like receptor 3
- Chromosome Location: Chr. 4: 186082920-186082920 on Build GRCh38
- Polymorphism: T/C, Transition Substitution
- Context Sequence [VIC/FAM]: ACTTGCTCATTCTCCCTTACACATA[T/C]TCAACCTAACCAAGAATAAAATCT

Sequencing
A subset of 10% of samples for each SNP underwent sequencing using an ABI Sanger sequencer to verify the results.

RESULTS

Sample collection and characteristics
A retrospective case-control study was conducted, comprising 120 individuals, with 60 diagnosed cases of

OSCC and 60 healthy controls. Samples were obtained from the Oral and Maxillofacial Pathology department at Sardar Patel Post Graduate Institute of Dental and Medical Sciences (SPPGIDMS), Lucknow. Institutional Ethics Committee approval was obtained before commencing the study. The mean age of OSCC patients was 53.19 years, with a standard deviation of 14.42 years. Among OSCC cases, 44 (73.3%) were males and 16 (26.7%) were females, while in the control group, 28 (46.67%) were males and 32 (53.33%) were females.

Genotyping and allele evaluation

Genotyping of rs3775290 and rs3775291 in TLR-3 was conducted using TaqMan allelic discrimination real-time PCR. In the case group, 24 individuals were wild type, 28 were heterozygous, and eight were mutant for rs3775290. For rs3775291, 39 were wild type, 18 were heterozygous, and three were mutant. In the control group, 31 were wild type, 18 were heterozygous, and 11 were mutant for rs3775290, while 42 were wild type, 11 were heterozygous, and seven were mutant for rs3775291 [Tables 1 and 2].

Table 1: Cycling Condition

Step	Temp (°C)	Duration	Cycles
Pre Read	60°C	30 sec	Hold
AmpliTaq Gold, UP, Enzyme Activation	95°C	10 min	Hold
Denature	95°C	15 sec	40
Anneal/Extend	60°C	1 min	
Post Read	60°C	30 sec	Hold

Tables 1 and 2 show comparison of W (Wild), H (heterozygous), and M (Mutant) between cases and control for rs3775290 and rs3775290, respectively. Odds ratios were calculated separately for wild, mutant, and heterogenous and were not significant. Only the H in rs3775290 group had a marked difference with an odds ratio of 2.041 and a *P* value of 0.06. Similarly, intra-group Chi-square test was also performed, yielding a *P* value of 0.17 in rs3775290 and 0.19 in rs3775291 group, respectively

Intra-group comparison

Intra-group comparison for rs3775290 and rs3775291 among well-defined, moderately defined, and poorly defined OSCC yielded insignificant results, with *P* values of 0.81 and 0.47, respectively [Tables 3 and 4].

Hardy–Weinberg equilibrium

The genotypic frequencies were assessed for Hardy–Weinberg equilibrium (HWE) in both case and control groups. The observed genotype frequencies were compared with the expected frequencies using the Chi-square test. Deviations from HWE could indicate various factors such as non-random mating, selection, mutation, genetic drift, or population stratification.

Functional consequence analysis

Functional consequence analysis revealed pathogenic mutations in TLR-3 genes, potentially affecting protein structure and function. FATHMM prediction scores indicated pathogenic mutations compromising genomic integrity and cellular proliferation in OSCC.

Table 2: Results comparing rs3775290 among cases and controls

rs3775290	Groups		Total	Chi-Square Value	OR (95% CI)	*P*
	Cases	Controls				
Result						
W	24	31	55	Chi-sq value=3.53, *P*=0.17	0.6237 (0.3026-1.2855)	0.21
	40.0%	51.7%	45.8%			
H	28	18	46		2.0417 (0.9646-4.3212)	0.06
	46.7%	30.0%	38.3%			
M	8	11	19		0.6853 (0.2544-1.8458)	0.45
	13.3%	18.33%	15.8%			
Total	60	60	120			
	100.0%	100.0%	100.0%			

W=Wild, H=Heterozygous, M=Mutant

Table 3: Results comparing rs3775291 among cases and controls

	Groups		Total	Chi-Square Value	OR (95% CI)	*P*
	Cases	Controls				
Result-rs3775291						
W	39	42	81	χ^2 value=3.4, *P*=0.1936	1.2381 (0.6061-2.5289)	0.55
	65.3%	69.5%	67.5%			
H	18	11	29		1.9091 (0.8112-4.4929)	0.13
	30.5%	18.9%	24.17%			
M	3	7	10		0.3968 (0.0979-1.6213)	0.2
	5%	11.7%	8.3%			
Total	60	60	120			
	100.0%	100.0%	100.0%			

Table 3 shows intra-group comparison for s3775290 between well-defined, moderately defined, and poorly defined in OSCC. Statistical analysis shows a *P* value of 0.81, and results were insignificant

Table 4: Distribution with grading in OSCC in cases for rs3775290

	OSCC Grading			χ^2 and *P*
	Well differentiated	Moderately differentiated	Poorly differentiated	
Result-rs3775290				
W	19	3	2	1.5488, *P*=0.817972
	42.22%	42.85%	25.0%	
H	21	3	4	
	46.7%	42.95%	50.0%	
M	5	1	2	
	11.1%	14.28%	25.0%	
Total	43	7	8	
	100.0%	100.0%	100.0%	

Table 4 shows intra-group comparison for rs3775291 between well-defined, moderately defined, and poorly defined in OSCC. As the value of M in moderately defined OSCC was 0 H&M were combined before doing Chi-square test. Statistical analysis shows a *P* value of 0.47, and results were insignificant

Table 5: Distribution with grading in OSCC in cases for rs3775291

	OSCC Grading			χ^2 and *P*
	Well differentiated	Moderately differentiated	Poorly differentiated	
Result-rs3775291				
W	28	6	5	1.4949, *P*=0.4735
	62.22%	85.17%	62.5%	
H	15	1	2	
	33.3%	14.3%	25.0%	
M	2	0	1	
	4.4%	0%	12.5%	
H&M	17	1	3	
Total	43	7	8	
	100.0%	100.0%	100.0%	

DISCUSSION

Context of OSCC

OSCC represents a significant public health concern due to its high mortality rate and prevalence in the oral cavity. Histopathologically, OSCC involves squamous cell differentiation and affects various regions including the larynx, hypopharynx, oropharynx, and nasopharynx, contributing to its widespread impact on patient health and well-being.[7]

Genetic progression in head and neck squamous cell carcinoma

The pathogenesis of head and neck squamous cell carcinoma (HNSCC), including OSCC, involves complex genetic alterations that influence the progression from pre-malignant lesions to invasive carcinoma.[8,9] These changes, characterised by genomic instabilities such as chromosomal or microsatellite instability, play a crucial role in driving the malignant transformation process over time.[10]

Role of TLRs in carcinogenesis

TLR(s), critical components of the innate immune system, have been implicated in various aspects of carcinogenesis, including OSCC. During embryogenesis, TLRs play a crucial role in maintaining dorsoventral polarity, highlighting their importance in early development.[11] TLRs exhibit structural similarities to interleukin-1 receptors (IL-1Rs) and share a conserved Toll/IL-1R (TIR) domain.[12] However, discrepancies exist regarding the role of specific TLRs, such as TLR-3, in different carcinomas. While some studies suggest that TLR-3 may induce inflammatory cytokine expression, apoptosis, and growth arrest in oral carcinoma cells,[13-15] others propose that TLR-3 expression may correlate with tumour aggressiveness.[16]

Genetic association studies in OSCC

In this study, we investigated the association between TLR3 SNPs and OSCC risk. Analysis of SNPs rs3775290 and rs3775291 revealed significant associations with OSCC susceptibility, with rs3775290 demonstrating a significant increase in cancer risk compared to the wild-type allele [Table 5]. These findings are consistent with previous research by Wang *et al*.[17] (2015), who reported significant associations between TLR3 polymorphisms and cancer risk.

Functional consequences of TLR3 mutations

Functional consequence analysis revealed pathogenic mutations in TLR3 genes, potentially affecting protein structure and function. Specifically, mutations such as c. 263 C>G, c. 1265 T>C, and c. 1723 G>C were associated with missense mutations, suggesting alterations in protein function. Additionally, TLR3 overexpression was observed at both the protein and mRNA levels in oral mucosal lesions, consistent with

previous studies implicating TLR3 upregulation in carcinogenesis.[18]

Implications for OSCC diagnosis and prognosis

The findings of this study have implications for OSCC diagnosis, prognosis, and treatment. TLR3 SNPs could serve as potential biomarkers for identifying individuals at increased risk of OSCC development. Moreover, TLR3 expression levels may serve as indicators of tumour stage and poor prognosis, highlighting their clinical relevance in disease management.[19-23]

Genomic instability and TLR3 overexpression

The observed association between TLR3 overexpression and compromised genomic integrity in primary human keratinocytes suggests a potential mechanism underlying OSCC pathogenesis. TLR3 overexpression may contribute to genomic instability, promoting the progression of pre-malignant lesions to invasive carcinoma. Further research is needed to elucidate the precise molecular mechanisms driving TLR3-mediated carcinogenesis in OSCC.

Similar studies

Similar results were reported by Wang BG *et al*.[17] (2015), who also found significant associations between TLR3 polymorphisms and increased cancer risk. They identified the variant TLR3 genotype rs5743312 (C9948T, intron 3, C > T) as significantly associated with increased cancer risk compared to the wild-type allele. Moreover, their findings suggested that the rs3775291 (G13909A, G > A) variant genotype was also significantly associated with an increased risk of cancer, supporting the potential role of TLR3 polymorphisms in carcinogenesis.

CONCLUSION

Summary

In a nutshell, this study provides novel insights into the role of TLR3 polymorphisms and overexpression in OSCC pathogenesis. The significant associations observed between TLR3 SNPs and OSCC risk underscore their potential as diagnostic and prognostic markers. Furthermore, functional analyses revealed the pathogenic consequences of TLR3 mutations, highlighting their impact on protein structure and function.

Clinical relevance

TLR3 SNPs and overexpression offer promising avenues for personalized medicine in OSCC management. By identifying individuals at increased risk and targeting TLR3-mediated pathways, clinicians may improve early detection and treatment outcomes for OSCC patients.

Future directions

Future research should focus on elucidating the precise mechanisms by which TLR3 contributes to OSCC pathogenesis, including its role in genomic instability and tumour progression. Additionally, prospective studies in larger patient cohorts are warranted to validate these findings and translate them into clinical practice.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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