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miRNA genetic variations associated with the predisposition of oral squamous cell carcinoma in central Indian population

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ABSTRACT

The disease burden of Oral Squamous Cell Carcinoma (OSCC) is rising day-by-day and is expected to rise 62 % through 2035. The chewing of tobacco, areca nut, and betel leaf, poor oral hygiene, and chronic infection are common risk factors of OSCC, but genetic and epigenetic factors also contribute equally. MicroRNAs (miRNAs) are comprised of small, non-coding endogenous RNA that regulate a plethora of biological activities by targeting messenger RNA through degradation or inhibition. Single Nucleotide Polymorphisms (SNPs) in miRNA genes can regulate the development and progression of OSCC. The present study aimed to determine the association between SNPs in miRNA genes (miRSNPs) with the risk of OSCC. A case-control study involving 225 histopathologically confirmed OSCC cases and 225 healthy controls was conducted, where 25 miRSNPs were analyzed by iPLEX MassArray analysis. A SNP rs12220909 in *MIR4293* showed a highly protective effect (*CC vs GG*, OR = 0.0431, 95%CI = 0.005–0.323, p = 3e-6). Whereas three SNPs, namely, rs4705342 in *MIR143* (*CC vs TT*, OR = 2.25, 95%CI = 2.00–2.53, p = 0.0008), rs531564 in *MIR124* (*CC vs GG*, OR = 24.18, 95%CI = 3.22–181.37, p = 3e-6), and rs3746444 in MIR499 (*AA vs GG*, OR = 2.01, 95%CI = 1.32–3.05, p = 0.001) were significantly associated with a higher risk of OSCC. Additionally, NanoString-based nCounter miRNA expression profiling revealed that miR-499a (Log2FC = −1.07), and miR-143 (Log2FC = −1.56) were aberrantly expressed in OSCC tissue. Taken together, the above miSNPs may contribute to the high incidence of OSCC in central India. However, further studies with large cohorts and ethnic stratification are required to validate our findings.

1. Introduction

GLOBOCAN reported an estimated 377,713 cases of lip and oral cavity cancer in the year 2020, accompanied by a global estimate of 177,757 deaths [1]. In India, oral cavity cancer lies in second position, with a total estimated cases of 135,929. Oral cancer is more prominent in Indian males and contributes to around 76.9 % of new cases [1]. Oral cancer arises as primary lesions on mucosal epithelium, and it is believed that 90 % belongs to oral squamous cell carcinoma (OSCC) [2]. Tobacco (smokeless/smoking), areca nut and betel leaf chewing, poor oral hygiene, chronic infection, and human papillomavirus are common risk factors of OSCC, but genetic and epigenetic factors also contribute equally [3]. Oral cancer statistics data from India revealed that age-adjusted rates are highest in males from the central zone, which is based on the Bhopal registry [4]. The reason for the high incidence of OSCC in the central zone (mainly Madhya Pradesh) of India is still not known.

MicroRNAs (miRNAs), 18–24 nucleotides long, small non-coding RNAs, are critical regulators of cellular processes such as differentiation, proliferation, metabolism, and cellular homeostasis. The seed sequence (2-mer to 8-mer) of miRNA binds to 3′ UTR/5’ UTR/CDS of target transcripts and regulates gene expression by translational inhibition of mRNA or by inducing cleavage of target mRNA [5]. miRNAs can act as tumor suppressor or oncogenic, depending on target mRNAs

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and their role in cancer development and progression. Genetic variations in miRNA genes, quantitative trait loci (QTL), and miRNA binding sites can directly or indirectly affect the stability of miRNAs or their binding to target mRNAs, thereby affecting protein expression and, consequently, OSCC pathogenesis [6]. SNPs in miRNA genes or their promoter regions lead to aberrant miRNA expression and ultimately affect the downstream target gene expression. Remarkable tissue specificity and dysregulation of several miRNAs have been observed in various cancer types, but no conclusive consensus has been achieved about the correlation of miRNA dysregulation with OSCC susceptibility [7]. Further, SNPs are specific to ethnicity; the observation in one population may not be followed in another due to variability in ancestral lineages of different countries. Therefore, understanding the effect of SNPs on cancer susceptibility is crucial for discerning the underlying molecular pathogenesis of various cancers.

Single nucleotide polymorphisms (SNPs) in miRNA genes and their abnormal expression in OSCC demand comprehensive research to provide ground for explaining the high incidence of OSCC in central India, which may lead to the development of screening tools for high-risk populations. Previously, we have shown that the CC genotype of rs2910164 *MIR146a* was significantly associated with the increased risk for OSCC in the central Indian population [8]. Therefore, we performed a case-control study to determine the role of 25 miRSNPs with the OSCC predisposition in the central Indian population.

2. Materials and methods

2.1. Study subjects

The present study was conducted at the All India Institute of Medical Sciences (AIIMS), Bhopal, which is located in the central zone (Madhya Pradesh) of India. A total of 450 participants, including 225 OSCC patients and 225 healthy controls from central India, were enrolled in the study. Newly diagnosed, histopathological confirmed adult cases of OSCC were included in the study. The enrolled control subjects were above 18 years old and tobacco consumers (smokeless tobacco) with at least a five-year consumption history. The study was approved by the Institutional Human Ethics Committee (IHEC), AIIMS Bhopal, and all participants signed the written informed consent. Venous blood (4 ml) was collected in the EDTA vials. A structured questionnaire was prepared and administered to all the participants about demographic details, family history of cancer, any previous history of chronic disease, tobacco, smoking, and alcohol drinking history, and other lifestyle habitats. The clinical characteristics are summarized in Table 1.

Table 1
Baseline characteristics of Subjects enrolled in the study.

Characteristic	Healthy, N = 225[a]	OSCC Patients, N = 225[a]	p-value[b]
Age	46.2(15.8)	48.7(13.1)	0.12
Gender	**% (number)**	**% (number)**	0.2
Female	21(47)	26 (58)	
Male	79 (178)	74 (167)	
Histopathology			
Moderately differentiated	NA	23	
Poorly differentiated	NA	45	
Well differentiated	NA	32	
Overall Staging			>0.9
I	NA	2.4	
II	NA	11	
III	NA	23	
IVA	NA	57	
IVB	NA	7.1	
History of Smoking			0.061
No	67	78	
Yes	33	22	
History of Tobacco chewing			<0.001
No	0	29	
Yes	100	71	
History of Alcohol			0.5
No	69	74	
Yes	31	26	
Family history of cancer			>0.9
No	NA	93	
Yes	NA	7.4	
Lesion site			>0.9
Alveolus	NA	11	
Buccal mucosa	NA	47.2	
Gingivobuccal sulcus	NA	2.4	
Hard palate	NA	1.2	
Tongue	NA	39	

[a] Mean(Standard deviation) or Frequency(%).
[b] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test.

2.2. microRNA SNPs (miRSNP) selection and genotyping

iPLEX® Assay and the MassARRAY® System (Agena MassARRAY®, San Diego, CA, USA) were used for genotyping. A total of 36 cancer-related miRSNPs (Supplementary Table S1) were selected, but only 25 SNPs were found compatible with iPLEX assay *in silico* designing with assay design software. The selection of miRSNPs was made by a thorough literature review and exploration of dbSNP, miRdSNP, miRSNP-v3, and OncomiR databases [9,10]. The SNPs selection criteria were: 1) the association of SNPs with cancer susceptibility, 2) SNPs in miRNA regions which alter expression, and target specificity. Forward, reverse, and extension primers were designed for each SNP using the Assay design software. Primers were manufactured by Integrated DNA Technologies (IDT, Singapore), and primer sequences are given in Supplementary Table S2. DNA was extracted with a modified phenol-chloroform method [11]. The quality and quantity of DNA were determined using a nano-spectrophotometer (ThermoFisher Scientific). A concentration of 10 ng/μl was used for the PCR reaction with iPLEX reagent kits. After PCR, the excess nucleotides were dephosphorylated by shrimp alkaline phosphatase, and mass-modified dideoxynucleotide terminators were added [12]. This was followed by the extension step, which used an extension primer specific for amplified fragments that anneal adjacent to SNP. The extended fragment is terminated by a single complementary base into the genotyping target site. The extension products were desalted by resins and dispensed into SpectroCHIP with an automated nanodispensor for MassArray analysis. The DNA detection range of the MassARRAY Analyzer DNA detection range is approximately 4500 Da to 9000 Da, and it also discriminates DNA with a difference of 16 Da. MassArray Typer software analyzed the data and provided automated allele calling [12].

2.3. Global miRNA expression profiling in OSCC

The significant miRSNPs showing significant association with OSCC were selected for further analysis. miRNA expression data were downloaded from The Cancer Genome Atlas Program (TCGA) Genomic Data Commons (GDC) with the GDC data transfer tool of the TCGA biolinks package (https://portal.gdc.cancer.gov/). The miRNA expression data covers patients with squamous cell carcinoma of the floor of the mouth, lip, oral cavity, base of tongue, palate, and gums. Multiple reads from different datasets were combined into a single read per million count, and each investigated miRNA read count was a fraction of the total miRNA count. miRNA expression normalization was done with quantile normalization of the limma package [13]. The significant (adjusted p-value <0.01) differences in miRNA expression profile between tumors of OSCC patients and normal samples were calculated using the limma package. The false discovery rates were determined with the Benjamini-Hochberg correction.

miR2Disease (www.mir2disease.org), OncomiR (www.oncomir.org), TargetScan (www.targetscan.org), miRDB (www.midb.org), and miRanda (www.microrna.org) databases were used to obtain the

predicted miRNA targets [14–16]. The predicted gene targets of miRNA were then analyzed with gene ontology (GO) Biological Process analysis using Enrichr (http://amp.pharm.mssm.edu/Enrichr/) [17]. Finally, we examined the correlation of miRNA expression and overall survival using Kaplan-Meier analysis and log-rank test using the R package 'survival' [18].

2.4. Clinical validation of miRNA signature

For the clinical validation of miRNA signatures retrieved from global *in silico* analysis, a total of six histo-pathologically confirmed cases of OSCC patients (Buccal mucosa; N = 6, Tongue; N = 6) were included in the study. The demographical and clinical details of the subjects under investigation are given in Table 2.

Surgical resection of tumors was done, and during the surgery, tumor tissue and normal tissue (2 cm. away from the tumor margin) were collected and placed in cryo-vials on liquid nitrogen immediately. The tissue samples were subsequently transferred to −80 °C deep freezer or liquid nitrogen for long-term storage. Total RNA was isolated from collected tissue samples using TRIzol reagent (Invitrogen), and Aurum™ RNA extraction kit (Bio-Rad Laboratories) [19]. RNA quantification and quality assessment were done spectrophotometrically (absorbance at 260, 230, and 280 nm) using a nano-spectrophotometer. miRNA expression profiling was performed with nCounter® gene expression panels with >800 miRNA targets (retrieved from mirBase) and five housekeeping transcripts [20]. Nanostring nCounter is a multiplex RNA hybridization assay that utilizes sequence-specific oligonucleotide reporter probes (tagged with fluorescent color code). These reporter probes bind to capture probe hybridized with mature miRNA and observed under nCounter digital analyzer. Sample integrity, quality, and background noise were determined with Positive and hybridization controls, negative proprietary spike-in controls, and ligation-specific controls. The data analysis was performed using nSolver software, and counts below 50 and with high variability (% CV > 90) were eliminated for the normalization of miRNA data. miRNA with significant SNPs were selected, and miRNA profiles between tumor and normal were performed. Small RNA sequencing was also performed using Illumina SP flow cells in NovaSeq 6000 instrument for six oral cancer patients with emphasis on miRNA-seq analysis. SncRNA libraries were prepared using the Qiaseq miRNA library kit, and sequencing was performed using combinatorial probe anchor synthesis (cPAS). small RNA seq data was trimmed by TrimGalore (https://github.com/FelixKrueger/TrimGalore) with a retaining of size more than 20. High-quality reads were to mature and available complementary star sequences on miRBase. miRNA counts were merged into a single matrix, and miRNA with a total sum count of less than 50 copies were removed from downstream analysis. Differentially expressed miRNAs were recognized by deseq2 R package, and miRNAs with false discovery rates less than 0.01were kept.

Table 2
Demographic and clinico-pathological details of OSCC patients included in miRNA expression profiling.

Factors	Buccal Mucosa, N = 6[a]	Tongue, N = 6[a]	P[b]
Gender			0.2
Female	0	50	
Male	100	50	
Staging			0.8
Stage-I	17	17	
Stage-II	17	50	
Stage-III	17	17	
Stage-IVA	50	17	
Histology			0.4
Invasive	0	17	
Moderately differentiated	33	67	
Well differentiated	50	17	
Well Differentiated	17	0	
Histology Grade			>0.9
Grade I	50	33	
Grade II	50	67	
Tumor Size			0.6
1 (2–4 cm)	33	67	
2 (>4 cm)	67	33	
Tumor Volume			0.6
1 (3–5 cm^3)	33	50	
2 (5–10 cm^3)	33	50	
3 (>10 cm^3)	33	0	
Tobacco History			0.4
No	0	40	
Yes	100	60	

[a] Mean (Standard deviation) or Frequency (%).
[b] Fisher's exact test.

2.5. Statistical analysis

The distribution of selected demographic and clinical factors based on genotype and allelic frequency between cases and controls was examined using the Pearson's Chi-square test, Wilcoxon rank sum test, and the Fisher's exact test. The Hardy–Weinberg equilibrium (HWE) was tested in the controls to assess any deviation of genotypic frequency with the goodness-of-fit test. Pearson Chi-square test was performed to calculate odds ratio (ORs), 95 % confidence interval (CI), and p-value with five different models: Allele, Dominant, Recessive, Homozygous, and Heterozygous. All statistical tests were carried out using SPSS-25 software (SPSS, Inc.) and RStudio. The tested hypothesis was defined as statistically significant with a p-value less than or equal to 0.005, and all the p-values are two-sided.

2.6. Theory

SNPs in miRNA genes have recently been identified as risk factor for various cancer including OSCC. The majority of studies are available with very less sample size and with a few SNPs, so we have attempted to study the role of 25 miRSNPs towards OSCC susceptibility in central Indian population with a sample size of 450 subjects. In addition, we also explored the miRNA expression profile of OSCC tumor tissue compared to the adjacent normal.

3. Results

3.1. Clinical characteristics of the study population

Of the 450 subjects, 225 were OSCC patients, and 225 were healthy subjects. The baseline characteristics of the enrolled subjects are shown in Table 1. There was no significant difference in the age of subjects between healthy controls and cases (p = 0.12); likely, the distribution of gender was also in equilibrium between the control and OSCC groups. Regarding the primary tumor sites in OSCC patients, buccal mucosa and tongue were the prominent anatomical sites of cancer, with 47 % and 39 %, respectively. The other sites of OSCC were alveolus (11 %), gingivobuccal sulcus (2.4 %), and hard palate (1.2 %). Overall staging was done as per the American Joint Committee on Cancer (AJCC) criteria. In our study, most of the OSCC patients were presented in advanced stage with clinical stage IV-A (57 %), followed by stage III (23 %), stage II (11 %), stage IVB (7.1 %), and stage I (2.4 %). All tumor samples were confirmed by histopathology, and the maximum proportion was of poorly differentiated (45 %) squamous cell carcinoma, with 32 % well-differentiated and 23 % moderately-differentiated. A majority (71 %) of OSCC patients had a history of consumption of smokeless tobacco. However, there was no significant difference in the status of smoking, tobacco chewing, and alcohol drinking between healthy and OSCC patients.

3.2. Allelic frequency of miRSNPs

Genotyping for 25 miRSNPs was performed with Agena MassARRAY,

and miRSNPs with a call rate of more than 90 % were considered for further statistical analysis. Out of selected 25 miRSNPs (Supplementary Table S1), the rs1834306 (*MIR110*, A/G), rs3501027 (*MIR196a2*, G/C), rs4938723 (*MIR34b*, T/C), rs6505162 (*MIR423*, C/A), and rs7227168 (*MIR4741*, C/T) were excluded from the investigation due to call rate lower than 90 %. The genotype call rate for 15 SNP assays was more than 90 %, and for five assays, the call rate was 100 %. Considering multiple comparisons, Bonferroni correction was applied, and a p-value of less than 0.002 was considered statistically significant. rs1143770 (*LET7a-2*, C > T) did not work in the central Indian population and, therefore, was not considered for further analysis. Four miRSNPs, rs12220909 (*MIR4293*), rs3746444 (*MIR499*), rs4705342 (*MIR143*), and rs531564 (MIR124) were found to be significantly associated with OSCC susceptibility ($p \leq 0.002$, Table 3). The observed genotyping frequency was in agreement with the expected Hardy-Weinberg equilibrium for all significant SNPs. The genotyping frequency distribution and significance level with odds ratio (OR) are given in Table 3. A statistically significant protective effect with the predisposition of OSCC was observed for rs12220909 (OR = 0.0431, 95 % CI = 0.005–0.323; p = 3e-6) (Table 3**).** However, rs4705342 (OR = 2.25, 95 % CI = 2.00–2.53; p = 0.0007), and rs531564 (OR = 24.18, 95 % CI = 3.22–181.37; p = 3e-6) were associated with increased risk of OSCC. While rs3746444 in the *MIR4*99 AA genotype was associated with OSCC risk (OR = 2.01, 95 % CI = 1.32–3.05; p = 0.001).

Furthermore, binary logistic regression was applied to avoid biases due to confounding factors like age and gender. The AA genotype of rs3746444 *MIR499* showed similar results after adjustment with age and gender (p < 0.001, OR = 2.33, 95 % CI = 1.523.56). Thereafter**,** the best predicted significant miRSNPs were analyzed for different genotyping models: Allele, Dominant, Recessive, Homozygous, and Heterozygous models. In the rs12220909 of *MIR4293*, individuals carrying the 'CC' genotype were on low OSCC risk compared to reference genotype 'GG' (*Dominant model;* OR = 0.157, 95 % CI = 0.05–0.46, p = 0.0001, *Recessive model;* OR = 0.04, 95 % CI = 0.005–0.32) (Table 5). Moreover, the rs1220909 was not significant in allele model that unveiled the recessive nature of the minor allele 'C'. In the rs3746444 in *MIR499a*, OSSC patients with at least one of the polymorphic allele 'G' tended to have lower OSCC risk as compared to reference allele 'A' (*AG vs GG*; OR = 0.13; 95 % CI = 0.05–0.31; p = 3e-8) (*AG + GG vs AA*; OR = 0.36; 95 % CI = 0.24–0.53; p = 4e-7) (*G vs A;* OR = 0.59; 95 % CI = 0.45–0.78, p = 0.0002). In the rs4705342 miRSNP, cases with the genotype 'CC' were on a higher risk for OSCC than the individuals having reference genotype 'TT' (*CC + TC vs TT*; OR = 6.81; 95 % CI = 2.96–15.65; p = 3e-7) (*CC vs TT*; OR = 2.25; 95 % CI = 2.00–2.53; p = 0.0007) (Table 4**).** Surprisingly, the minor allele 'C' was significantly associated with OSCC risk in the heterozygous model (*TC vs TT;* OR = 5.19; 95 % CI = 2.21–12.17; p = 3e-5), but not in the allelic model. In the rs531564 of *MIR124*, the minor allele 'C' was associated with elevated OSCC risk (*GC vs CC*; OR = 24.35; 95 % CI = 3.07–192.67; p = 3e-5) (*CC vs GG + GC*; OR = 24.21; 95 % CI = 3.23–181.27; p = 3e-6) (*C vs G*; OR = 2.4; 95 % CI = 1.55–3.70; p = 6e-5) (Table 4). In the rs3746444 miRSNP, patients with AA allele were at high risk for developing OSCC (*AA vs GG*; OR = 2.01; 95 % CI = 1.32–3.05; p = 0.001) (*AA vs GG + AG*; OR = 2.77; 95 % CI = 1.85–4.14; p = 4e-7) (Table 3). The A allele of rs3746444 was also significantly associated with OSCC risk (*A vs G*; OR = 1.66; 95 % CI = 1.27–2.17; p = 0.0002) (Table 3). We then investigated the correlation between miRNA polymorphisms, clinical pathological parameters, and risk factors of OSCC patients. Nevertheless, the allelic genotypic distribution of miRSNP did not affect the status of clinical stage, tumor site, tumor size, and histological grade of OSCC patients **(**Supplementary Table S3**).**

3.3. *In silico analysis of miRNA significantly associated with OSCC susceptibility*

The miRNAs chosen from the previous objective (miR-4293, miR-499, miR-143, and miR-124) were assessed for expression analysis in OSCC samples with TCGA datasets (GDC data portal). TCGA datasets of oral cancer (till July 5, 2023) were retrieved and analyzed with TCGAbiolinks, clusterprofiler, edgeR, and EDASeq package (RStudio). We downloaded the miRNA-Seq data of selected 332 TCGA datasets (https://portal.gdc.cancer.gov/), and miRNA expression was counted by Transcripts per million (TPM). Data was normalized and filtered with a 0.25 quantity cutoff, and differential expression was performed. Then, we assessed the expression profile of the miRNA under investigation (Fig. 1A). The significantly differentially expressed miRNAs were miR-499a (Log2FC = −3.16, p-value = 4.66E-19), and miR-143 (Log2FC = −1.2, and p-value = 6.79E-10). miR-499 and miR-143 were significantly downregulated in the tumors from OSCC patients. However, miR-4293 and miR-124 were not significantly dysregulated in the available TCGA data. ENCORI (Encyclopedia of RNA interactions) based pan-cancer survival analysis shows a significant correlation between lower expression of miR-499-5p (p-value = 0.00012) and miR-143-3p (p-value = 0.016) with OS of head and neck squamous cell carcinoma (HNSCC) patients with hazard ratio 1.71, and 1.40, respectively (Fig. 1C). The patients with low expression of miR-499-5p and miR-143-3p exhibited better prognosis (overall survival) of OSCC patients compared to those with high expression. Further, the *in silico* expression profile of miR-499-5p and miR-143-3p was validated in the central India population with NanoString-based nCounter assay.

3.4. *Validation of miRNA expression profile in OSCC patients with nCounter assay by NanoString*

Agena MassArray-based genotyping and *in silico* differential expression analysis of miRNAs from OSCC patients reveals miR-499a-5p and miR-143-3p as key miRNAs. To validate the expression of these differentially expressed miRNAs in the central India population, an nCounter

Table 3
Association of miRNA genetic polymorphism and OSCC susceptibility.

S. No.	miRSNP	Genotype	OSCC cases		Control		p-value	Odd ratio with confidence interval (CI)
			No.	Percent	No.	Percent		
1	miR-4293 (rs12220909 G > C)	GG	221	98.3	200	89.7	*Ref*	
		CC	1	0.40	21	9.5	3e-6	0.0431 (0.005–0.323)
		GC	3	1.30	2	0.8	1.00	1.35 (0.22–8.20)
2	miR-124 (rs531564 G > C)	GG	171	77.38	188	85.45	*Ref*	
		CC	22	99.95	1	0.45	3e-6	24.18 (3.22–181.37)
		GC	28	12.67	31	14.1	1	0.99 (0.57–1.72)
3	miR499a (rs3746444 A > G)	AA	106	47.32	55	24.44	*Ref*	
		GG	111	49.56	116	51.56	0.001	0.49 (0.33–0.75)
		AG	7	3.12	54	24.0	0	0.06 (0.02–0.15)
4	miR-143 (rs4705342 T > C)	TT	171	4.52	211	0.47	*Ref*	
		CC	10	80.91	1	96.2	0.0008	2.25 (2.00–2.53)
		TC	29	14.57	7	3.33	3e-5	5.19 (2.21–12.17)

Table 4
Association of miRNA genetic polymorphism and increased OSCC risk in different genotyping models.

Gene Polymorphism	OSCC patients	Healthy control	Corrected Chi-square	Odds Ratio (95 % CI)	Fisher Exact 2 Tailed P value
miR499a (rs3746444 A > G)					
Dominant Model					
(AA + AG) vs GG	50/50	48.4/51.6	0.054	1.06 (0.73–1.54)	0.7
(GG + AG) vs AA	52.7/47.3	75.5/24.5	24.55	0.36 (0.24–0.53)	4e-7
Heterozygote Model					
AG vs AA	6.3/93.7	49.5/50.5	50.16	0.06 (0.02–0.15)	0
AG vs GG	5.9/94.1	31.7/68.3	26.31	0.13 (0.05–0.31)	3e-8
Recessive Model					
AA vs GG + AG	47.3/52.7	24.4/75.6	24.55	2.77 (1.85–4.14)	4e-7
GG vs AA + AG	49.5/50.5	51.5/48.5	0.05	0.93 (0.64–1.36)	0.7
Allele					
A vs G	48.8/51.2	36.4/63.6	13.69	1.66 (1.27–2.17)/	0.0002
G vs A			0.59 (0.45–0.78)	0.0002	
Homozygotes Model					
AA vs GG	48.9/51.1	25.4/74.6	10.28	2.01 (1.32–3.05)	0.001
GG vs AA	51.1/48.9	74.6/25.4	10.28	0.49 (0.32–0.75)	0.001
miR-143 (rs4705342 T > C)					
Dominant Model					
(TT + TC) vs CC	95.4/4.6	99.5/0.5	7.68	0.95 (0.92–0.98)	0.001
(CC + TC) vs TT	19.1/80.9	3.8/96.2	24.17	6.81 (2.96–15.65)	3e-7
Heterozygous Model					
TC vs TT	15.2/84.8	3.3/96.7	15.78	5.19 (2.21–12.17)	3e-5
TC vs CC	76.3/23.7	87.5/12.5	0.35	0.46 (0.04–4.25)	0.31
Recessive Model					
TT vs CC + TC	80.9/19.1	96.1/3.9	22.1	0.14 (0.06–0.33)	3e-7
CC vs TT + TC	4.5/95.5	0.5/99.5	5.42	4.52 (1.63–7.40)	0.001
Allele					
T vs C	11.8/88.2	2.1/97.9	32.26	0.12 (0.05–0.28)	0
C vs T				7.86 (3.50–17.61)	0
Homozygotes Model					
TT vs CC	94.7/5.3	99.5/0.5	8.83	0.44 (0.39–0.49)	0.0007
CC vs TT	5.3/94.7	0.5/99.5	8.83	2.25 (2.0–2.53)	0.0007
miR-124 (rs531564 G > C)					
Dominant Model					
(GG + GC) vs CC	90.0/10.0	99.5/0.5	18.25	0.04 (0.005–0.30)	3e-6
(CC + GC) vs GG	22.6/77.4	14.5/85.5	4.23	1.717 (1.052–2.803)	0.03
Heterozygous Model					
GC vs GG	14.1/85.9	14.1/85.9	0	0.993 (0.572–1.723)	1
GC vs CC	56/44	96.8/0.2	14.19	24.35 (3.07–192.67)	3e-5
Recessive Model					
GG vs CC + GC	77.3/22.7	85.4/14.6	4.23	0.582 (0.356–0.949)	0.03
CC vs GG + GC	9.9/90.1	0.5/99.5	18.25	24.21 (3.23–181.27)	3e-6
Allele					
G vs C83.7/16.3	92.5/7.5	15.41	0.416 (0.269–0.644)	6e-5	
C vs G				2.4 (1.55–3.70)	6e-5
Homozygotes Model					
GG vs CC	88.6/11.4	99.5/0.5	18.06	0.041 (0.005–0.31-0.31)	3e-6
CC vs GG	11.4/88.6	0.5/99.5	18.06	24.187 (3.225–181.371)	3e-6

assay was performed in paired samples (tumor tissues and adjacent normal tissues) from six OSCC patients having tumors in the buccal mucosa (N = 3), and tongue (N = 3). Our laboratory data was in concordance with *in silico* findings, following the same pattern of downregulation of miR-499a-5p and miR-143-3p with Log2FC −1.07, and −1.56 (Fig. 1B). The correlation of clinical features (TNM staging, OSCC staging, histology grade, and tumor volume) and miRNA expression are shown in Fig. 2 miR-143-3p dysregulation showed an association with tumor volume ($p = 0.03$). However, no significant correlation was observed between the selected clinical features and the expression of other miRNAs. In continuation of NanoString-based expression profiling, we also performed small RNA-Seq in a different set of patients with OSCC (N = 6). The findings revealed the significant downregulation of miR-499a-5p in tumors with Log2FC −4.66 (P-adjusted value = 0.04). Similarly, miR-143-3p was downregulated in tumor tissue with Log2FC −1.36 (P-adjusted value = 0.05), which represents the similar pattern of miRNA expression as in NanoString data. This further validated the NanoString expression analysis with adhesion to the homogenous pattern of selected miRNAs.

4. Discussion

Oral cancer incidence in central India populations (Madhya Pradesh) is very high. In India, it is the leading cancer in men and the fifth most common cancer in women. Likewise, Bhopal, the capital of Madhya Pradesh, also shares the highest cancer cases among male cancer patients (17 %) (Population Based Cancer Registry of Bhopal) [4]. The common risk factors found among the central Indian population are smokeless tobacco chewing and lifestyle. In addition, owing to the multifactorial origin of oral cancer, genetic background and polymorphism contribute to the genetic predisposition to cancer. Previous findings suggest a role of SNPs with genetic predisposition of oral cancer in several ethnicities [20,21]. The role of miRSNPs in the genetic predisposition of OSCC in the Indian population has not been well studied. This is the first MassArray-based genotyping study with selected miRSNPs in the Indian population, which explored the association of miRSNPs with OSCC predisposition. In the present study, we found genotypes, CC of rs12220909 *MIR4293*, CC of rs531564 *MIR124*, AA of rs3746444 *MIR499a*, and CC of rs4705342 *MIR143* are significantly associated with OSCC susceptibility ($p \leq 0.005$). *In silico* expression analysis of these miRNAs revealed the dysregulation of miR-499 and

Table 5
Association of miRNA genetic polymorphism and lower risk of OSCC in different genotyping models.

Gene Polymorphism	OSCC patients	Healthy control	Corrected Chi-square	Odds Ratio (95 % CI)	Fisher Exact 2 Tailed P value
miR-4293 (rs12220909 G > C)					
Dominant Model					
(GG + GC) vs CC	99.0/1.0	90.5/9.4	17.43	23.28 (3.09–174.09)	3e-6
(CC + GC) vs GG	1.8/98.2	10.3/89.6	12.94	0.157 (0.05–0.462)	0.0001
Heterozygote Model					
GC vs GG	1.4/98.6	1/99	0	1.35 (0.22–8.2)	1
GC vs CC	75/25	8.6/91.4	6.01	31.5 (2.14–463.16)	0.01
Recessive Model					
GG vs CC + GC	98.2/1.8	89.6/10.4	12.94	6.35 (2.16–18.68)	0.0001
CC vs GG + GC	0.5/99.5	9.4/90.6	17.43	0.04 (0.005–0.32)	3e-6
Allele					
G vs C	98.8/1.2	90.1/9.9	31.53	9.74 (3.82–24.80)	0
Homozygous Model					
GG vs CC	99.5/0.5	90.4/9.6	17.35	23.20 (3.09–174.09)	3e-6
CC vs GG	0.5/99.5	9.5/90.5	17.35	0.04 (0.005–0.323)	3e-6

miR-143 in OSCC tumors compared to adjacent normal tissues. Further, a similar set of miRNA expression was also analyzed in NanoString nCounter assay-based miRNA expression profile OSCC patients of central India, which showed a similar pattern of downregulation of miR-499 and miR-143. Downregulation of miR-499a and miR-143 was also confirmed by RNA-Seq analysis. However, no significant deregulation was observed in the expression pattern of miR124 (Fig. 1A) in the TCGA data set or in OSCC patients of central India.

In our study, the AA genotype of rs3746444 (A > G) SNP of *MIR499a* is significantly associated with the increased risk of OSCC in the central Indian population. rs3746444 SNP is present in the seed sequence of *MIR499a* and, therefore, has a higher probability of affecting miRNA function and, thereby, miRNA-based target gene silencing [22]. Multiple meta-analysis studies revealed the association of rs3746444 with cancer risk in several cancers, including breast cancer [23], cervical squamous cell carcinoma, hepatocellular carcinoma [24], lung cancer, and prostate cancer [25]. Ge et al., 2019 demonstrated the correlation of SNP rs3746444 with NSCLC between malignant pulmonary nodules and benign pulmonary nodules groups [26]. They revealed the significant association of the AA genotype with NSCLC risk. Also, they found that the expression levels of miR-499a in the AA group were much higher than in the GG group [26]. rs3746444 of *MIR499a* polymorphism is located in chromosome 20q11.22, and it has been shown to destabilize the pre-miR499a structure [27]. Furthermore, a study has shown that this structural deformation affects the expression of SOX genes that subsequently activate the Wnt/β-catenin signaling pathway [28].

The rs4705342 (T > C) is present in the promoter of miR-143/miR-145 gene cluster located on chromosome 5q32, and our study demonstrates a significant correlation of rs4705342 (T > C) polymorphism with OSCC risk in the central Indian population. The CC genotype of rs4705342 was correlated with high OSCC risk in the recessive and homozygote models. The rs4705342 is an important polymorphism and has been proven to be a functional variant in prostate cancer and CSCC with different transcriptional activity [29,30]. Zhao et al., 2023 observed the high risk of epithelial ovarian cancer (EOC) with CC genotype (p = 0.01) and correlation with the disease in progression-free survival and overall survival of EOC patients (HR = 1.30, 95 % CI = 1.04–1.62, P = 0.020; HR = 1.33, 95 % CI = 1.05–1.70, P = 0.020) [31]. In our study, the frequency of the C allele was significantly higher in OSCC patients and further correlated with tobacco consumption (p < 0.03). Our study summarized that the CC genotype might significantly reduce the expression of miR-143 in OSCC tissues, and tobacco consumers are more prone to have this miRSNP. The rs4705342 T > C of *MIR143* is proven functional polymorphism in many studies, and the rs4705342T allele showed a higher protein-binding affinity but lower promoter activity, and the rs4705343C allele showed a reduced transcriptional activity [32]. In our study, miR-143 aberrant expression was significantly correlated with the tumor volume (p = 0.03).

rs12220909 is present in the seed region of miR-4293 [33]. A few previous studies could not find an association between rs12220909, and the risk of nasopharyngeal cancer, breast cancer, and hepatocellular carcinoma. However, in our study, we found a significant association between the genotype CC and reduced OSCC risk. Similarly, some studies demonstrated the decreased risk of ESCC and NSCLC in the Chinese population [34,35].

The rs531564 of *MIR124* is a very common polymorphism in the pre-miR-124-1, and has been shown to affect the expression of mature miR-124 [36]. In our study, rs531564 was found to be significantly associated with an increased risk of OSCC (*CC vs GG*, OR = 24.18, 95 % CI = 3.22–181.37, P = 3e-6). The polymorphic genotype 'CC' was more prominent in the OSCC population in comparison to healthy control, with 99.95 % and 0.45 %, respectively. The same observation was observed in the Xuzhou Han Chinese population, in which the pri-miR-124 rs531564 polymorphism was significantly linked with the reduced risk of colorectal cancer (*GG vs. CC*: OR = 0.25, 95%CI = 0.09–0.67, P = 0.003 (*CG + GG) vs. CC*: OR = 0.73, 95%CI = 0.56–0.94, P = 0.01); [37]. A meta-analysis explored the association between rs531564 with breast and cervical cancer risk and found the genotype 'GG' reduces the risk of female neoplasm compared to the population with 'CC' (OR = 0.41, 95%CI = 0.27–0.61, P = 0.01) or women carrying at least one C allele (OR = 0.72, 95%CI = 0.53–0.99, P = 0.04) [38].

Taken together, our study showed a significant association of rs12220909 *MIR4293*, rs531564 *MIR124*, rs3746444 *MIR499*, and rs4705342 *MIR143* with OSCC predisposition. The expression profile demonstrated the downregulation of miR-499a and miR-143 in OSCC patients and a significant correlation between miR-143 dysregulation and tumor volume (p = 0.03). SNPs in pre-miRNA/prompter disrupt the secondary structure to promote or demote the miRNA maturation/expression, while SNPs in mature miRNA alter target binding specificity and affinity [39]. These functional alterations in tumor suppressor or oncogenic miRNA may lead to dysregulation of miRNA expression and, ultimately, increase the propensity to develop OSCC. Overall, the SNP iPLEX MassArray is an established and high throughput technology but is limited by the number of SNPs and special requirements of instrument. The small sample size for miRNA expression analysis is the main limitation of the present study, which limits the exploration of the effect of miRSNPs on miRNA expression. Although our findings are promising, further studies are warranted with a larger sample size to interpret results in the highly sensitive association of selected miRSNPs with OSCC risk.

5. Conclusion

In conclusion, our study examined the relationship between 25 miRSNPs and OSCC predisposition. The genotype CC of rs12220909 MIR4293 is associated with a lower risk of OSCC, while CC of rs531564 MIR124, AA of rs3746444 MIR499, and CC of rs4705342 MIR143 increase the risk of OSCC in central India population. Concurrently, expression of miR-499a, and miR-143 were downregulated in tumor tissues from OSCC patients. However, further research in this field is warranted to perform a comprehensive evaluation and complete understanding of carcinogenesis in larger cohorts, stratified by ethnicity, to better recognize the association between miRNA polymorphism and

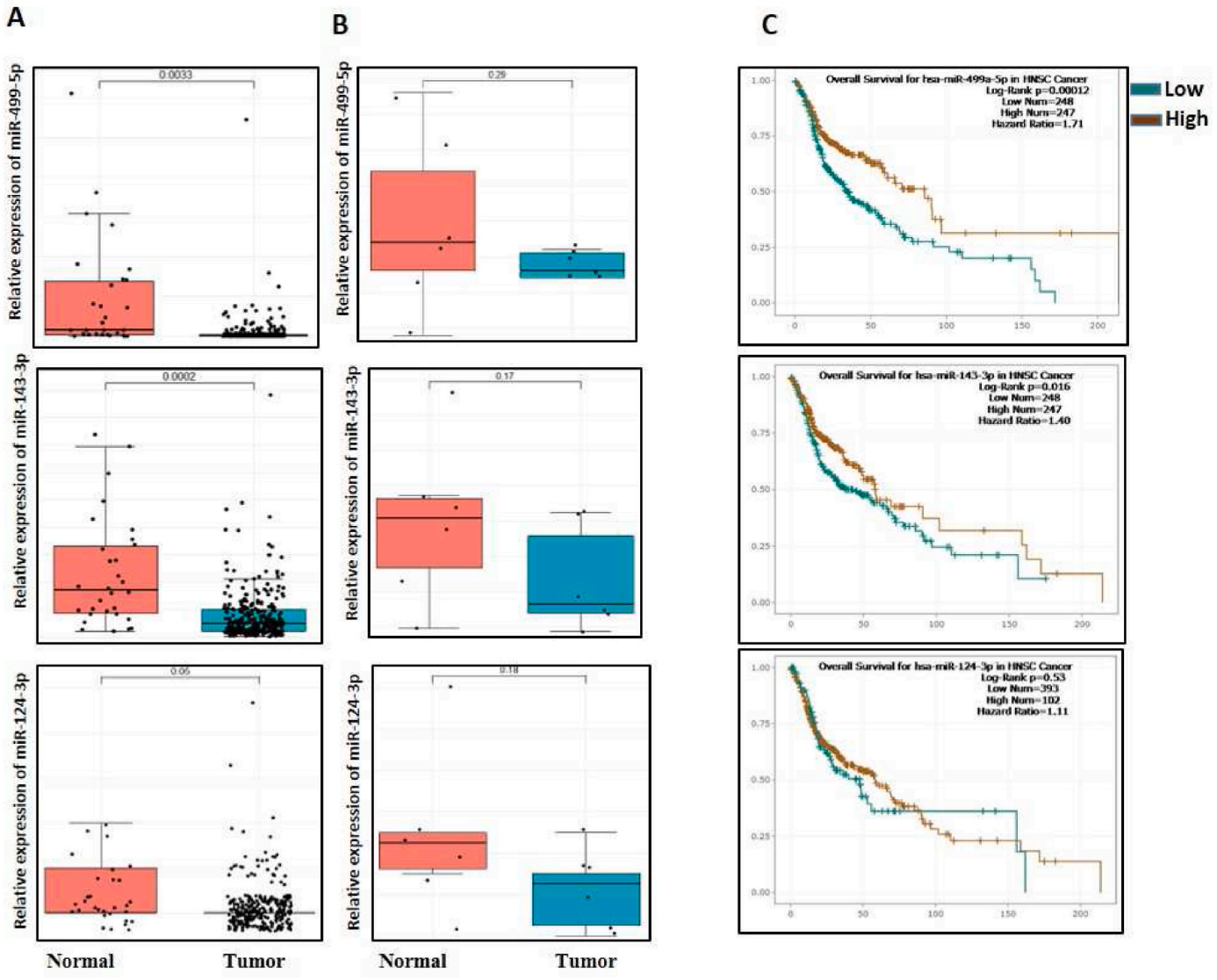

Fig. 1. (A) Expression analysis of miR-499-5p, miR-143-3p, and miR-124-3p. (A, B, C) Expression of miR-499-5p (p-value = 0.0033), miR-143-3p (p-value = 0.0002), and miR-124-3p (p-value = 0.05) decrease significantly in OSCC tumors, compared to normal tissue.
(B) NanoString based expression analysis of miR-499-5p, miR-143-3p, and miR-124-3p. Expression of miR-499-5p (p-value = 0.29), miR-124-3p (p-value = 0.19), miR-143-3p (p-value = 0.17) downregulate in OSCC tumors, compared to normal tissue.
(C) Overall survival analysis of miR-499-5p, miR-143-3p, and miR-124-3p. A-C) Overall survival of head and neck squamous cell carcinoma patients with upregulation and downregulation of miR-499-5p (p-value = 0.00012), miR-124-3p (p-value = 0.53), and miR-143-3p (p-value = 0.016). Patients with low expression of miR-499-5p, and miR-143-3p exhibits better prognosis (overall survival) of OSCC patients compared to patients with high expression.

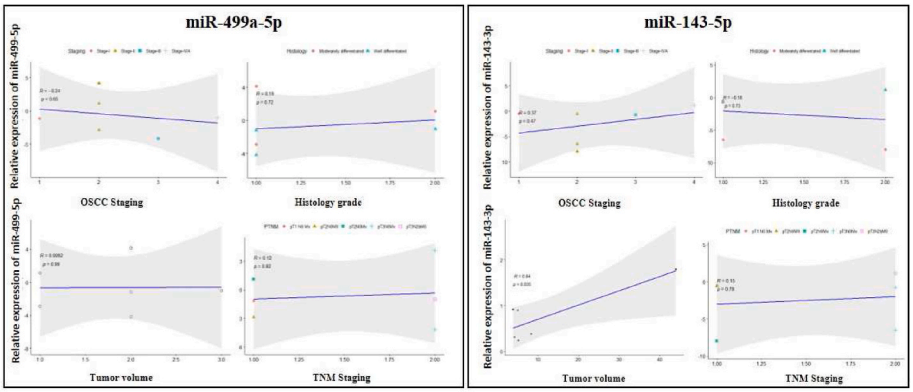

Fig. 2. Correlation analysis of miR-499-5p, miR-143-3p, and miR-34b-3p with OSCC staging, histology grade, tumor volume, and TNM Staging. **A- D)** miR-499-5p; **E-H)** miR-143-3p.

OSCC risk. Furthermore, functional validation studies of miRSNPs on the effects of miRNA levels, stability, or binding with their target mRNAs are also required.

Disclosure for conflict of interest

Authors declare that there is no conflict of interest.

CRediT authorship contribution statement

Shikha Tiwari: Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ritu Pandey:** Resources, Methodology. **Vinay Kumar:** Supervision, Resources. **Saikat Das:** Visualization, Supervision, Resources. **Vikas Gupta:** Resources. **Rajeev Nema:** Supervision, Conceptualization. **Ashok Kumar:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ncrna.2024.07.002.

References

[1] A. Miranda-Filho, F. Bray, Global patterns and trends in cancers of the lip, tongue and mouth, Oral Oncol. 102 (2020) 104551, https://doi.org/10.1016/j.oraloncology.2019.104551.
[2] P. Sushma, K. Jamil, P.U. Kumar, U. Satyanarayana, M. Ramakrishna, B. Triveni, Genetic variation in microRNAs and risk of oral squamous cell carcinoma in South Indian population, Asian Pac. J. Cancer Prev. APJCP 16 (2015) 7589–7594.
[3] L. Nokovitch, C. Maquet, F. Crampon, I. Taihi, L.-M. Roussel, R. Obongo, et al., Oral cavity squamous cell carcinoma risk factors: state of the art, J. Clin. Med. 12 (2023) 3264.
[4] S. Sharma, L. Satyanarayana, S. Asthana, K.K. Shivalingesh, B.S. Goutham, S. Ramachandra, Oral cancer statistics in India on the basis of first report of 29 population-based cancer registries, J. Oral Maxillofac. Pathol. 22 (2018) 18–26, https://doi.org/10.4103/jomfp.JOMFP_113_17.
[5] M. Vahabi, G. Blandino, S. Di Agostino, MicroRNAs in head and neck squamous cell carcinoma: a possible challenge as biomarkers, determinants for the choice of therapy and targets for personalized molecular therapies, Transl. Cancer Res. 10 (2021) 3090–3110, https://doi.org/10.21037/tcr-20-2530.
[6] T. Arancibia, S. Morales-Pison, E. Maldonado, L. Jara, Association between single-nucleotide polymorphisms in miRNA and breast cancer risk: an updated review, Biol. Res. 54 (2021) 26, https://doi.org/10.1186/s40659-021-00349-z.
[7] J. Zeng, X. Yi, H. Liu, Y. Yang, Y. Duan, H. Chen, Polymorphisms in four microRNAs and risk of oral squamous cell cancer: a meta-analysis, Oncotarget 9 (2018) 8695–8705, https://doi.org/10.18632/oncotarget.24211.
[8] S. Tiwari, R. Pandey, V. Kumar, S. Das, V. Gupta, S. Vishwakarma, et al., Association of single nucleotide polymorphism miRNA-146a (rs2910164) with increased predisposition to oral squamous cell carcinoma in central India population, Cancer Biomarkers 38 (2023) 203–214, https://doi.org/10.3233/CBM-230064.
[9] C.-J. Liu, X. Fu, M. Xia, Q. Zhang, Z. Gu, A.-Y. Guo, miRNASNP-v3: a comprehensive database for SNPs and disease-related variations in miRNAs and miRNA targets, Nucleic Acids Res. 49 (2020) D1276–D1281, https://doi.org/10.1093/nar/gkaa783.
[10] A.E. Bruno, L. Li, J.L. Kalabus, Y. Pan, A. Yu, Z. Hu, miRdSNP: a database of disease-associated SNPs and microRNA target sites on 3'UTRs of human genes, BMC Genom. 13 (2012) 44, https://doi.org/10.1186/1471-2164-13-44.
[11] D.K. Lahiri, J.I.J. Nurnberger, A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies, Nucleic Acids Res. 19 (1991) 5444, https://doi.org/10.1093/nar/19.19.5444.
[12] S. Gabriel, L. Ziaugra, D. Tabbaa, SNP genotyping using the Sequenom MassARRAY iPLEX platform, Current Protocols in Human Genetics 60 (2009) 2–12.
[13] M.E. Ritchie, B. Phipson, D. Wu, Y. Hu, C.W. Law, W. Shi, et al., Limma powers differential expression analyses for RNA-sequencing and microarray studies, Nucleic Acids Res. 43 (2015) e47, https://doi.org/10.1093/nar/gkv007, e47.
[14] A.J. Enright, B. John, U. Gaul, T. Tuschl, C. Sander, D.S. Marks, MicroRNA targets in Drosophila, Genome Biol. 5 (2003) R1, https://doi.org/10.1186/gb-2003-5-1-r1.
[15] Q. Jiang, Y. Wang, Y. Hao, L. Juan, M. Teng, X. Zhang, et al., miR2Disease: a manually curated database for microRNA deregulation in human disease, Nucleic Acids Res. 37 (2009) D98–D104, https://doi.org/10.1093/nar/gkn714.
[16] V. Agarwal, G.W. Bell, J.-W. Nam, D.P. Bartel, Predicting effective microRNA target sites in mammalian mRNAs, Elife 4 (2015), https://doi.org/10.7554/eLife.05005.
[17] M.V. Kuleshov, M.R. Jones, A.D. Rouillard, N.F. Fernandez, Q. Duan, Z. Wang, et al., Enrichr: a comprehensive gene set enrichment analysis web server 2016 update, Nucleic Acids Res. 44 (2016) W90–W97.
[18] T.M. Therneau, A Package for Survival Analysis in R, 2023.
[19] S. Vishwakarma, R. Agarwal, S.K. Goel, R.K. Panday, R. Singh, R. Sukumaran, et al., Altered expression of sphingosine-1-phosphate metabolizing enzymes in oral cancer correlate with clinicopathological attributes, Cancer Invest. 35 (2017) 139–141.
[20] H. Rezazadeh, F. Rezazadeh, M.J. Mokhtari, M.J. Fattahi, M.A. Semnani, others, Evaluation of the Association of Mir-143 and Rs4705342 Single Nucleotide Polymorphism with Oral Squamous Cell Carcinoma, 2023.
[21] C. Liu, W. Gao, Y. Shi, L. Lv, W. Tang, Association between miR-146a rs2910164, miR-196a2 rs11614913, and miR-499 rs3746444 polymorphisms and the risk of esophageal carcinoma: a case–control study, Cancer Med. 11 (2022) 3949–3959.
[22] W. Chen, D. Shao, H. Gu, J. Gong, J. Zhang, Hsa-mir-499 rs3746444 T/C polymorphism is associated with increased risk of coronary artery disease in a Chinese population, Acta Cardiol. Sin. 33 (2017) 34–40, https://doi.org/10.6515/acs20160303a.
[23] S.C. Tan, P.Y. Lim, J. Fang, M.F.M. Mokhtar, E.A.M. Hanif, R. Jamal, Association between MIR499A rs3746444 polymorphism and breast cancer susceptibility: a meta-analysis, Sci. Rep. 10 (2020) 3508.
[24] J.-K. Jiang, H.-S. Chen, W.-F. Tang, Y. Chen, J. Lin, Rs3746444 T> C locus in miR-499 increases the susceptibility to hepatocellular carcinoma: a meta-analysis 14812 subjects, World J. Gastrointest. Oncol. 15 (2023) 171.
[25] X. Yang, X. Li, B. Zhou, A meta-analysis of miR-499 rs3746444 polymorphism for cancer risk of different systems: evidence from 65 case-control studies, Front. Physiol. 9 (2018) 737.
[26] N. Ge, C. Mao, Q. Yang, B. Han, Y. Wang, L. Xu, et al., Single nucleotide polymorphism rs3746444 in miR-499a affects susceptibility to non-small cell lung carcinoma by regulating the expression of CD200, Int. J. Mol. Med. 43 (2019) 2221–2229, https://doi.org/10.3892/ijmm.2019.4124.
[27] B.M. Ryan, A.I. Robles, C.C. Harris, Genetic variation in microRNA networks: the implications for cancer research, Nat. Rev. Cancer 10 (2010) 389–402.
[28] X. Li, J. Wang, Z. Jia, Q. Cui, C. Zhang, W. Wang, et al., MiR-499 regulates cell proliferation and apoptosis during late-stage cardiac differentiation via Sox6 and cyclin D1, PLoS One 8 (2013) e74504.
[29] H. Chu, D. Zhong, J. Tang, J. Li, Y. Xue, N. Tong, et al., A functional variant in miR-143 promoter contributes to prostate cancer risk, Arch. Toxicol. 90 (2016) 403–414.
[30] Y. Liang, R. Sun, L. Li, F. Yuan, W. Liang, L. Wang, et al., A functional polymorphism in the promoter of MiR-143/145 is associated with the risk of cervical squamous cell carcinoma in Chinese women: a case–control study, Medicine 94 (2015).
[31] J. Zhao, W. Zuo, Y. Zhang, C. He, W. Zhao, T. Meng, The polymorphism rs4705342 in the promoter of miR-143/145 is related to the risk of epithelial ovarian cancer and patient prognosis, Front. Oncol. 13 (2023) 1122284.
[32] R. Liu, H. Fu, Y. Yu, Q. Xu, J. Fang, Q. Ge, et al., Association of miR-4293 rs12220909 polymorphism with cancer risk: a meta-analysis of 8394 subjects, Medicine (Baltim.) 99 (2020) e21364, https://doi.org/10.1097/MD.0000000000021364.
[33] H. Danesh, M. Hashemi, F. Bizhani, S.M. Hashemi, G. Bahari, Association study of miR-100, miR-124-1, miR-218-2, miR-301b, miR-605, and miR-4293 polymorphisms and the risk of breast cancer in a sample of Iranian population, Gene 647 (2018) 73–78, https://doi.org/10.1016/j.gene.2018.01.025.
[34] P. Zhang, J. Wang, T. Lu, X. Wang, Y. Zheng, S. Guo, et al., miR-449b rs10061133 and miR-4293 rs12220909 polymorphisms are associated with decreased esophageal squamous cell carcinoma in a Chinese population, Tumour Biol 36 (2015) 8789–8795, https://doi.org/10.1007/s13277-015-3422-2.
[35] L. Fan, L. Chen, X. Ni, S. Guo, Y. Zhou, C. Wang, et al., Genetic variant of miR-4293 rs12220909 is associated with susceptibility to non-small cell lung cancer in a Chinese Han population, PLoS One 12 (2017) e0175666, https://doi.org/10.1371/journal.pone.0175666.
[36] L. Qi, Y. Hu, Y. Zhan, J. Wang, B.-B. Wang, H.-F. Xia, et al., A SNP site in pri-miR-124 changes mature miR-124 expression but no contribution to Alzheimer's disease in a Mongolian population, Neurosci. Lett. 515 (2012) 1–6, https://doi.org/10.1016/j.neulet.2012.02.061.

[37] X. Gao, H. Wang, S. Zhang, M. Wang, Z. Zhu, Pri-miR-124 rs531564 polymorphism and colorectal cancer risk, Sci. Rep. 5 (2015) 14818, https://doi.org/10.1038/srep14818.
[38] M. Bastami, J. Choupani, Z. Saadatian, S. Zununi Vahed, E. Ouladsahebmadarek, Y. Mansoori, et al., Evidences from a systematic review and meta-analysis unveil the role of MiRNA polymorphisms in the predisposition to female neoplasms, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20205088.
[39] G. Sun, J. Yan, K. Noltner, J. Feng, H. Li, D.A. Sarkis, et al., SNPs in human miRNA genes affect biogenesis and function, RNA 15 (2009) 1640–1651, https://doi.org/10.1261/rna.1560209.