Differential Roles of $p16^{INK4A}$ and $p14^{ARF}$ Genes in Prognosis of Oral Carcinoma

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Abstract

Background: Oral cancer patients are found to have poor clinical outcome and high disease recurrence rate, in spite of an aggressive treatment regimen. The inactivation of INK4A/ARF loci is reported to be second to p53 inactivation in human cancers. The purpose of this study was to assess the prognostic significance of the molecular aberrations in the INK4A locus for effective identification of aggressive oral carcinoma cases needing alternate therapy.

Materials and Methods: The study composed of 116 patients freshly diagnosed with oral carcinoma. The genetic and epigenetic status of the $p16^{INK4A}$ and $p14^{ARF}$ genes was evaluated. The relation between these genic alterations and different treatment end points, such as residual disease (initial response), disease recurrence, and overall survival, along with the standard clinical markers, were analyzed.

Results: 62% of the study cases had $p16^{INK4A}$ gene abnormalities, with deletion accounting for 33% and methylation for 29%. Alterations in $p14^{ARF}$ gene either by deletion (12%) and/or methylation (18%) were observed in 30% of the cases. $p16^{INK4A}$ deletion was associated with aggressive tumors, as evidenced by the nodal involvement of the disease. Low or absence of $p16^{INK4A}$ protein adversely affected the initial treatment response. Promoter methylation of $p16^{INK4A}$ was associated with increased disease recurrence and acts as an independent predictor for worse prognosis. Surprisingly, $p14^{ARF}$ methylation associated with lower recurrence rate in oral cancer patients with a good clinical outcome. Overall survival of these patients was associated with tumor size, nodal disease, and $p16^{INK4A}$ protein expression pattern. Our results indicate that $p16^{INK4A}$ and $p14^{ARF}$ alterations constitute a major molecular abnormality in oral cancer cases.

Conclusion: The molecular profile of INK4A/ARF locus, both at DNA and protein level, could be used as a prognostic biomarker for assessing the aggressiveness of disease in oral carcinoma patients. The study further shows the opposing clinical effect of these two genes, transcribed from the same locus, in oral cancer patients.

Introduction

Head and neck squamous cell carcinoma represents the sixth most common cancer worldwide and accounts for 500,000 new cases annually. In some European and Southeast Asian countries, including India, oral cancer prevails to be the leading form of cancer (1, 2). In oral carcinoma cases, poor survival outcome with a probability of survival at 5 years being <50% is seen, in spite of adopting the most aggressive multimodal treatment strategies (3). High recurrence rate leading to treatment failure seems to be a major drawback in the patient treatment protocol. The current cornerstone of therapeutic decision-making is based on the tumor-node-metastasis system supplemented with conventional histopathologic tumor grading, which has proved to be unsatisfactory prognostic indicators in the present scenario. Determination of the biological behavior and identification of prognostic biomarkers is important for the early detection of relapse, as well as for stratification of patients before enrollment onto their treatment regimen.

The INK4A locus, harboring the $p16^{INK4A}$ gene, is a major aberration hotspot in oral carcinoma. The $p16^{INK4A}$ gene functions as negative regulator of the cell cycle progression through its inhibition of cdk4/6 and subsequent blockage of the cyclin-dependent phosphorylation of the Rb (4). Genetic alterations of $p16^{INK4A}$ lead to loss of control of the restriction point in the G1 phase of the cell cycle and favor cellular transformation (5). In our earlier study, it is found that $p16^{INK4A}$ expression loss defines a subgroup of oral cancer patients with worse clinical outcome (6). The present study aimed at deciphering the molecular alterations involved in $p16^{INK4A}$ down-regulation and to assess the prognostic implications of $p16^{INK4A}$ gene alterations in oral carcinoma. As instability of this locus is reported to be high in carcinoma, we analyzed $p16^{INK4A}$ and its alternate spliced product—$p14^{ARF}$ at molecular level. The $p14^{ARF}$ tumor suppressor gene has a unique first exon that
splices into exons 2 and 3 of p16\(^{INK4A}\) gene in a different reading frame, thus translating a distinct protein that bears no amino acid homology to the p16\(^{INK4A}\) gene product. p14\(^{ARF}\) acts by binding to mdm2, a negative regulator of p53, causing stabilization of p53 and leading to cell cycle arrest (7).

Major inactivation of the p16\(^{INK4A}/p14^{ARF}\) genes results from promoter methylation, homozygous deletion, and intragenic mutation. These genetic and epigenetic alterations have been detected frequently in a variety of human cancers, including head and neck cancer (8, 9). The alteration affecting the \(INK4A/ARF\) locus can potentially disrupt the two main tumor suppression pathways, p16\(^{INK4A}\)-Rb and p14\(^{ARF}\)-p53. In this study, we did a comprehensive genetic and epigenetic analysis of both the genes to identify whether these genes can be used as a prognostic biomarker in oral carcinoma. The results indicate p14\(^{ARF}\) and p16\(^{INK4A}\) genes to have strong clinical implications but with differential roles in oral carcinoma.

Materials and Methods

**Patient Characteristics and Tissue Sample.** The present study population of 116 oral cancer patients was selected from our original cohort of 348 patients previously described (6). Availability of tumor tissue for DNA isolation was the criteria for selecting the present cohort from our original one. Information on patient characteristics, both personal and clinical, is given in Table 1. From each patient, an incision biopsy was collected from the lesion and divided into two bits. One bit was immediately snap frozen in liquid nitrogen and stored at \(-80^\circ\) C for molecular work-up, and the other bit was used for histopathologic reporting and immunohistochemical analyses. The histopathologic grading and staging of the lesion were done as per the WHO criteria (10). The institutional Review Board approved the experimental design, specimen collection procedure, and scope of this study. Mean age of the patients at diagnosis was 59 years (range, 36-85 years). Genderwise, 82 cases (71%) were male and 34 (29%) were female. More than 60% of the patients were presented with extensive lesion at the time of diagnosis. Radiation alone or in combination with surgery was the main mode of treatment given. Clinical follow-up for 50 months was conducted with a median follow-up of 23 months.

**DNA Extraction.** The genomic DNA from tumor and normal samples were extracted by standard phenol-chloroform method. Briefly, tissues were pulverized and treated with proteinase K followed by phenol-chloroform extraction. Ethanol precipitation was carried out, and DNA was dissolved in Tris-EDTA buffer (pH 8.0).

**Homzygous Deletion Analysis.** To assess the homozygous deletion status of these two genes, competitive multiplex PCR was carried out. Each exon of p14\(^{ARF}\) and p16\(^{INK4A}\) was amplified with a fragment of either \(\beta\)-actin or GADPH as internal controls (11-13). Plasmid and blood DNA from healthy volunteers were used as normal controls. The PCR reaction mixture consisted of 1 × PCR buffer [10 mmol/L Tris (pH 8.3), 50 mmol/L KCl], 200 μmol/L deoxynucleotide triphosphate, 1.5 mmol/L MgCl₂, primers (0.15-0.3 μmol/L), 1 unit Taq polymerase, 2.5% DMSO, and 80 to 100 ng of genomic DNA. PCR was done for 30 to 35 cycles having annealing temperature of 58 to 60°C for 1 min. The PCR products were separated on agarose gel, and ethidium bromide-stained fragments were recorded by the gel...
Methylation of INK4A/ARF Locus. The methylation pattern of the CpG islands of p16\textsuperscript{INK4A} and p14\textsuperscript{ARF} promoters were determined by bisulfite modification of the genomic DNA followed by methylation-specific PCR (14). Briefly, 2 μg of DNA were denatured with NaOH (3 mol/L) at 37°C for 15 min. After denaturation, 5.6 mol/L sodium metabisulfite (pH 5) and 10 mmol/L hydroquinone were added and incubated at 55°C for 16 h. The samples were desalted using DNA Clean-Up System (Promega). NaOH (0.3 mol/L) was used for alkali desulfonation at 37°C for 15 min. Neutralization of sample was carried out using 3 mol/L sodium acetate followed by ethanol precipitation, and DNA was resuspended in TE buffer. Bisulfite-converted blood DNA from normal healthy individuals served as negative controls. Placental DNA treated with MSssl methyltransferase (New England Biolabs) was used as positive control for methylated alleles. To ensure the reproducibility of the results, all samples were analyzed twice.

The promoter methylation profiles of p14\textsuperscript{ARF} and p16\textsuperscript{INK4A} were determined using primers specifically designed for either methylated or unmethylated sequence (14, 15). PCR was carried out using 1× PCR buffer [10 mmol/L Tris (pH 8.3), 50 mmol/L KCl], 1 to 1.5 mmol/L MgCl\textsubscript{2}, deoxynucleotide triphosphate (200 μmol/L each), methylated or unmethylated specific primers (0.5 μmol/L each), 1 unit of Taq DNA polymerase, and ~50 ng of bisulfite-modified DNA in a total volume of 25 μL. The PCR amplification process was carried out for 35 to 40 cycles with annealing at 64°C (methylation) or 60°C (unmethylation). PCR products were resolved in agarose gel, and the bands were visualized by ethidium bromide staining.

Mutation Analysis. The point mutations in p14\textsuperscript{ARF} and p16\textsuperscript{INK4A} genes were screened using PCR single-strand conformational polymorphism technique for exon 1p (p14\textsuperscript{ARF}, exon 1a (p16\textsuperscript{INK4A}), and exon 2 individually. The primers used for PCR single-strand conformational polymorphism analysis were the same as used in deletion analysis. Samples showing band shift were further subjected to direct DNA sequencing to confirm and characterize the mutation. Sequencing was done in automated ABI Prism 377 DNA sequencer with the ABI Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) according to manufacturer’s instructions.

Treatment End Points. Out of 116 study subjects, 113 patients were subjected to radical therapy. Out of 113 treated cases, clinically, the disease completely disappeared in 80 patients (71.4%). In 32 patients (28.6%), residual disease persisted even after the completion of the treatment. Out of the 80 patients that showed complete remission of the disease, 29 cases (37.2%) showed recurrence during follow-up. Whereas in 49 patients (62.8%), no recurrence was observed up to the end of the follow-up period. Irrespective of disease status, 47 treated patients (43.9%) were alive up to last follow-up and 60 patients (56.1%) died during the follow-up period. Thus, three end points were considered for the analysis, namely, residual disease, disease recurrence, and overall survival. The data of patients who were lost to follow-up during the study period were treated as unavailable data when assessing the prognostic outcomes.

Data Analysis. The association between the categorical variables was assessed using χ\textsuperscript{2} test. Statistical analyses between various variables and survival end points were carried out using χ\textsuperscript{2} analysis for residual disease and log-rank test for disease recurrence and overall survival. Variables found significant in the univariate analysis were analyzed together in multivariate analysis using logistic regression for residual disease and Cox’s proportional hazard regression analysis for disease recurrence and overall survival to find out their independent influence over the dependent variable and to calculate its risk, hazard ratio (HR). The Cox model was adjusted for potential confounding by age and gender. A two-tailed P value of <0.05 was considered as statistically significant.

Results

Methylation Analysis. The methylation status of the promoter region of p16\textsuperscript{INK4A} and p14\textsuperscript{ARF} genes was investigated in 116 cases. Thirty-four samples (29.3%) were positive for p16\textsuperscript{INK4A} methylation and 21 cases (18.1%) showed p14\textsuperscript{ARF} promoter methylation. Representative gels are shown in Fig. 1. Concomitant methylation of both the genes was present in seven samples (6%).

INK4A/ARF Deletion Status. Deletion analysis was carried out in all the four exons (exons 1a, 1p, 2, and 3). Deletion of at least one of the three exons of p16\textsuperscript{INK4A} was observed in 38 cases (32.8%), and of p14\textsuperscript{ARF}, only 14 cases (12.1%) showed deletion (Fig. 2). The frequencies of exonwise deletions were 17.8% for exon 1a, 9% for exon 1p, 2.8% for exon 2, and 8.3% for exon 3. In 12 cases (10.3%), simultaneous deletion of exons either common to (2 and 3) or specific to (1a and 1p) p16\textsuperscript{INK4A} and p14\textsuperscript{ARF} were noted.

Mutation Detection. Among the oral cancer samples analyzed for mutation, only one case showed an altered migration pattern in single-strand conformational polymorphism analysis of exon-1a of p16\textsuperscript{INK4A} gene. The sequencing of the single-strand conformational polymorphism–positive sample confirmed a C→T transition at codon 38, altering the codon triplet CCC (Pro)→TCC (Ser). No mutation was observed in other exons including the p14\textsuperscript{ARF}–specific exon 1p.

Gene Inactivation. By combining all these molecular alterations together, the genes were scored as either inactivated or not. If any of the deletion, methylation, or mutation was positive in the exons specific to a particular gene, then that gene was considered as inactivated in that sample. Totally, the p16\textsuperscript{INK4A} gene inactivation was seen in 62.2% of cases, whereas p14\textsuperscript{ARF} inactivation was observed only in 30.1% of oral cancer cases.

Relation between Clinicopathologic Factors and Molecular Alterations. To compare the p16\textsuperscript{INK4A} inactivation
methylated, whereas S23 showed hypermethylation of p14ARF gene inactivation did not relate to any of the clinicopathologic variables. No association was found between p14ARF and p16INK4A expression pattern, alone is associated with residual disease, it independently favored residual

Figure 1. Methylation detection of p16INK4A and p14ARF gene using methylation-specific PCR. A. p14ARF promoter hypermethylation analysis in agarose gel. Tumor sample S11 is unmethylated, whereas S23 showed hypermethylation of p14ARF. B. p16INK4A methylation analysis showed S22 to be p16INK4A methylated. U, unmethylated lanes; M, methylated lanes; MSSs, methyltransferase treated placenta; P, normal placenta; S, tumor samples.

Prognostic Significance

Univariate Analysis. The clinicopathologic variables (T status, N status, stage, and histopathology) and the genic alterations (methylation, deletion, mutation, inactivation of p14ARF/p16INK4A genes, and p16INK4A protein expression) were analyzed with various treatment end points. Variables showing significant association with treatment end points are shown in Table 2. The presence of residual disease was associated only with the p16INK4A protein expression pattern (P = 0.037). No other clinical or experimental variables analyzed show any association with the presence of residual disease. Around 84% of the lesions with expression of p16INK4A protein showed good initial treatment response, and no residual disease was present in these cases.

Log-rank analyses revealed that the disease recurrence is significantly related to N status (P = 0.001), methylation status (P = 0.013) of p16INK4A, and also with the methylation (P = 0.030) and gene inactivation status (P = 0.011) of p14ARF gene. Among the p16INK4A methylated cases, 54% of cases developed recurrence during the follow-up period. More than thrice increased relative risk (RR) was observed for patients with hypermethylation of p16INK4A gene to develop recurrence (RR = 3.3). However, oral carcinoma patients had nine times lower risk in developing recurrence when p14ARF was methylated (RR = 0.109). Kaplan-Meier curve for recurrence-free survival and the methylation status of these two genes are given in Fig. 3A and B. The seven samples having concomitant methylation of p14ARF and p16INK4A showed complete recurrence-free survival (P = 0.011). The deletion status of p14ARF and p16INK4A did not relate to disease recurrence. When p14ARF gene inactivation was analyzed by taking together both the methylation and deletion status, it was found to have a significant effect on recurrence, and the chance for developing recurrence was eight times lower for p14ARF inactive patients (RR = 0.130; Fig. 3C). However, disease recurrence was not significantly associated with p16INK4A gene inactivation. The clinical variable-nodal involvement also showed a high significant relation with the disease recurrence. But the risk is comparatively very low (RR = 1.32) than for the molecular variables.

The overall survival showed significant association with clinical variables, such as T status (P = 0.049), N status (P = 0.001), and p16INK4A protein expression (P = 0.016). No other molecular markers showed any relation with overall survival. The RR for clinical variables, such as tumor size (Fig. 3E) and presence of nodes (Fig. 3F) were 1.8 and 1.7, respectively. However, the absence of p16INK4A protein expression confers comparatively a high risk (3.1) for poor overall survival in oral cancer patients (Fig. 3D).

Multivariate Analysis. Because only one factor, the p16INK4A protein expression pattern, alone is associated with residual disease, it independently favored residual

Figure 2. Deletion analysis. A. p16INK4A exon 1a deletion in lanes 3, 7, and 8. B. p14ARF exon 1b deletion in lanes 2, 4, and 5. C. Exon 2 deletion in third and fourth lane. D. p16INK4A exon 3 deletion in lane 4. β-Actin and GADPH as reference genes. Lane M, marker; lane 1, normal sample; other individual lanes, oral carcinoma samples.
Molecular Analysis of INK4A/ARF Locus in Oral Cancer

Table 2. The variables showing significant relation with residual disease, disease recurrence, and overall survival by univariate analysis in oral carcinoma patients

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<td>0.318</td>
<td>0.136 - 0.745</td>
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disease in oral carcinoma patients. Cox’s regression analysis showed that $p16^{INK4A}$ methylation (HR = 2.4) and active $p14^{ARF}$ gene, i.e., unmethylated or undeleted $p14^{ARF}$ (HR = 11) were independently associated with the disease recurrence. With overall survival, all the factors that were significant in univariate analysis, such as lesion size (HR = 1.325), nodal involvement (HR = 1.520), and loss of $p16^{INK4A}$ protein expression (HR = 1.98) were significant in the multivariate model as independent factors. Variables found significant in multivariate analysis are shown in Table 3. The Kaplan-Meier survival curves of the factors independently influencing the disease recurrence and overall survival are shown in Fig. 3. Thus, when the prognostic outcomes were taken into account, advanced lesions, presence of nodal disease, low $p16^{INK4A}$ protein expression, and $p16^{INK4A}$ methylation served as independent adverse prognostic markers. Whereas $p14^{ARF}$ inactivation, including $p14^{ARF}$ methylation, was found to be a good prognostic predictor for oral carcinoma.

![Figure 3](image-url)

*Figure 3.* Kaplan-Meier survival curves of various factors that showed independent influence on recurrence free and overall survival of oral cancer patients. **A.** $p16^{INK4A}$ methylation and recurrence-free survival. **B.** $p14^{ARF}$ methylation and recurrence-free survival. **C.** $p14^{ARF}$ gene inactivation and recurrence-free survival. **D.** $p16^{INK4A}$ protein expression and overall survival. **E.** T status and overall survival. **F.** N status and overall survival.
provides evidence that the genes, assessed for their prognostic influence. The present study categorized as an early event as not seen with late observation is in contrast to previous reports linking overexpression confirming the functional inactivation of p16INK4A. Many reports have associated p16INK4A methylation as an early event in oral carcinogenesis. In the present study, although methylation of p16INK4A was observed in only 0.5% of the T1 lesions, it is difficult to categorize it as an early event as no trend is seen with late stage tumors. Also the T1 cases were very few (7%) in the sample population.

Our present study observed only a single case with point mutation, and it was localized in the exon-1a region of p16INK4A gene. Such low frequency of somatic mutation in p16INK4A gene has been reported in earlier studies (20). A total deletion frequency of 33% was observed for p16INK4A, with exon 1a (18%) being the main target for deletion. Interestingly, two cases showed complete loss of all the three exons of p16INK4A, whereas four other patients exhibited either exon 2 or exon 3 deletion concomitant with exon 1 deletion. Nodal involvement leading to tumor aggressiveness is another hallmark of p16INK4A inactive oral cancer cases (9). In this study also, we found a significant association between the nodal involvement and homozygous deletion of p16INK4A gene. The up-regulation of the proliferative genes regulated by the transcription factor like elk-1 after p16INK4A inactivation could be a factor for the tumor aggressiveness (21). Although p16INK4A deletion and gene inactivation did not associate with protein expression status statistically, 57% and 63% cases showed absence of protein expression, respectively. The homozygous deletion/inactivation of p16INK4A did not have an effect on the prognostic outcome of oral carcinoma cases. The overall survival of patients was influenced by nodal disease, increased tumor size (status), and p16INK4A protein down-regulation. Two times increased risk for poor survival was observed for patients with loss of p16INK4A protein expression.

The tumor suppressor gene p14ARF was inactivated in 30% of oral cancer patients and was significantly associated with recurrence-free survival of patients. Low frequency of p14ARF homozygous deletion (12%) was observed in the present study, consistent with previous reports (22). Deletion status of p14ARF was not significantly linked to any variables analyzed. However, p14ARF methylation was strongly associated with low disease recurrence in oral carcinoma with 9-fold decreased risk for recurrence when compared with unmethylated cases. When p14ARF gene inactivation was considered, the risk increased to 11 times. This shows that, the p14ARF deletion also influenced the recurrence rate in combination with its promoter methylation. This observation is in contrast to previous reports linking p14ARF methylation with poor prognosis (23). A study by Ogi et al. is the only available report that observed an association between p14ARF methylation and better survival in carcinoma cases but they were unable to arrive at a conclusion for such a result (24). A recent report on glioma supports the role of p14ARF as modulator for radioresistance (25). As radiation is the main mode of treatment in our patients, the absence of p14ARF might be rendering the tumor cells more sensitive to radiation. The increased radiosensitivity in p14ARF inactive patients might enhance the prognosis of the patients. Here, we hypothesize that p14ARF might be playing some role in treatment modulation leading to better prognostic outcome. p14ARF nuclear overexpression showed an increased growth fraction, aggressive clinical course, and shortened survival in B-cell lymphoma (26). This

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*Adjusted for age and gender.

Discussion

Oral squamous cell carcinoma is characterized by local tumor aggressiveness and high recurrence rate. The pursuit of molecular markers capable of predicting the prognosis of oral carcinoma patients is compelling as clinically similar tumors may display different response to therapy. Loss of p16INK4A protein proved to be an independent factor in predicting poor survival in these patients. As p16INK4A was altered in a large number of oral carcinoma cases, the genetic alteration of p16INK4A along with its alternate spliced product (p14ARF) were assessed for their prognostic influence. The present study provides evidence that the genes, p16INK4A and p14ARF, transcribed from the INK4A loci significantly influence the disease prognosis in oral carcinoma patients, and these genes seem to function in diametrically opposite directions.

Loss of p16INK4A in the Rb pathway releases the inhibition in CCND1/cdk complex, which in turn phosphorylates Rb, leading to cell cycle progression (16). The p16INK4A gene inactivation (deletion and/or methylation) was observed in 62% of cases in our study. In the present study, aberrant methylation of p16INK4A was found in 29% of oral carcinoma samples, consistent with previous reports (17). The methylation profile of p16INK4A significantly correlated with loss of protein expression confirming the functional inactivation of p16INK4A. The tumorsuppressor gene p14ARF was inactivated in 30% of oral cancer patients and was significantly associated with recurrence-free survival of patients.
postulates p14\(^{ARF}\) as a surrogate marker for highly deregulated cell cycle with aggressive tumors. In our analysis also, the p14\(^{ARF}\) active (unmethylated) patients had a poor clinical course. Unfortunately, we were unable to carry out the expression analysis of p14\(^{ARF}\) in our study population due to nonavailability of samples.

Concomitant methylation of p14\(^{ARF}\) and p16\(^{INK4A}\) was observed in seven patients. When these cases were analyzed, none of the patients showed disease recurrence. It remains unclear how hypermethylation of p14\(^{ARF}\) and p16\(^{INK4A}\) contribute to good prognosis in these patients. The absence of p14\(^{ARF}\) could be giving an advantage during the treatment even in the absence of p16\(^{INK4A}\) tumor suppressor. Evidence supports hypermethyltion of p14\(^{ARF}\) and p16\(^{INK4A}\) to be independent events, and each seems to contribute differentially during carcinogenesis (15).

Evidences from the present study surprisingly show that p14\(^{ARF}\) and p16\(^{INK4A}\) differ in their functionality in determining disease recurrence in oral carcinoma patients. Our previous study showed that the loss of expression of p16\(^{INK4A}\) was significantly associated with poor outcome in oral cancer patients treated with radiation (27). Simon et al. showed p16\(^{INK4A}\) expression as radiosensitizer, whereas p14\(^{ARF}\) expression rendered radioresistance in glioma cell lines (25). Our observation also points to such an inference, supporting a role for p16\(^{INK4A}\) and p14\(^{ARF}\) as modulators of radioresistance in oral cancer. The methylation status of p14\(^{ARF}\) and p16\(^{INK4A}\) genes could be valuable prognostic biomarker in identifying patient subgroups with increased risk for disease recurrence. On the other hand, p16\(^{INK4A}\) protein expression influenced the initial treatment response and overall survival of patients. However, further studies on a larger cohort are needed to validate the present findings.

Alltogether, our findings point to the significant effect ofINK4A/ARF locus displaying differential effects on the prognosis of oral carcinoma patients. To the best of our knowledge, no other study has observed a contrasting role for p14\(^{ARF}\) and p16\(^{INK4A}\) methylation in association with recurrence-free survival in cancer patients. Although both p16\(^{INK4A}\) and p14\(^{ARF}\) are transcribed from the same locus, their inactivation does not seem to be functionally equivalent in oral carcinoma. This suggests that the methylation status of these genes should be considered individually before the initiation of treatment for a better prognosis. The early detection of aberrant methylation of these genes may assist in identifying specific patients who might benefit from alternate therapeutic intervention.

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References
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