Association of *Aurka* Gene Polymorphism (*RS2064863*) with Oral Squamous Cell Carcinoma Development - An *In Silico* Study

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ABSTRACT

Background: Oral carcinoma is the most common and sixth cause of death worldwide. *AURKA* gene encodes Aurora kinase-A, cell-cycle regulated protein which is involved in microtubule formations and stabilization of spindle poles during chromosome segregation. Overexpression of the gene is often associated with chromosome instability.

Objective: The study investigates the association of *AURKA* gene polymorphism in oral squamous cell carcinoma (OSCC) progression.

Methods: The present study follows an observational study design which employs *in-silico* tools to assess the frequency of the alleles related to the polymorphism (*rs2064863*) in different populations and its possible consequences.

Results: The polymorphism (*rs2064863*) of *AURKA* gene selected was an intron variant with the minor allele frequency of 0.37 for the G allele. Interestingly, the ancestral population or the African population demonstrated a frequency of 83% for the T allele and 16% for the G allele, where other populations such as American, European and South Asian populations presented with different allele frequencies in which both the alleles occur with similar frequencies in the population.

Conclusion: The present study revealed the possible reasons for the positive selection of the minor allele in these populations, which could provide clues on the possible association of this variant with OSCC in the south Asian population.

Key Words: *AURKA*, Oral Squamous Cell Carcinoma, Polymorphism, Aneuploidy, Positive selection, Allele frequencies

INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is the sixth most common cause of death worldwide. More than 90% of all head and neck tumors occur in OSCC patients. Treatment at an advanced stage of SCC has a relatively low survival rate of five years. Both genetic factors and carcinogen exposure like alcohol and tobacco regulate SEC development. Poor diet and physical inactivity have also been known to contribute Carcinoma.¹ It has been demonstrated by previous studies that genetic polymorphism combined with betel nut carcinogens increases the susceptibility to OSCC development.² OSCC is a lethal deforming disease with tumor innovation costing or facial destruction and cervical lymph node metastasis. Globally, there is an incident of 300,000 new cases a year with a significant rise in young adults.³ Metabo-

lomics can provide insight into the treatment and prognosis of OSCC.⁴ The sub-site of oral cancer has decreased therapeutic outcome in spite of high modes of treatment.⁵ Field cancerization is used to describe the recurrence of OSCC after excision of primary tumors.⁶ Myofibroblasts play a crucial role in OSCC development as they help in disease progression.⁷ The final saliva is contributed by oral mucosa, periodontium, and oral microbial flora which can be used as a diagnostic tool in OSCC detection.⁸ Salivary metabolomics also aids in identifying tumor-specific biomarkers which in turn helps in the detection of tumor progression.⁹ Regional spread of carcinoma is commonly seen in OSCC.¹⁰

Aurora kinase is a serine/threonine kinase with 3 groups, Aurora A, Aurora B, and Aurora C. Aurora A and Aurora B have a central role in mitosis while Aurora C has a unique role

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in meiosis. Gene amplification of aurora kinases has been reported in human malignancies which signify their potential role as oncogenes in tumorigenesis. Aurora kinase can be used as a target in anticancer therapeutics.¹¹ *AURKA* is a centrosome related gene which is overexpressed in human cancers. High expression of *AURKA* leads to centrosome amplification, chromosome segregation, and aneuploidy resulting in malignant transformation.²

MATERIALS AND METHODS

One of the intron variants (*rs2064863*) of the *AURKA* gene was selected for the study based on the literature mining process. Since oral cancer is a disease that is more predominant in patients with a chronic history of using smoking and smokeless tobacco such as pan, gutka, etc., genetic variants that were more closely related to environmental factors were identified in the gene and selected. A recent study by Chou et al, 2017 demonstrated that patients with *rs2064863* polymorphism were at a 1.365-fold risk of OSCC progression when compared to those with the wild-type allele. The Ensembl database was used to acquire the frequency data of the variant in different populations (11). Furthermore, the expression of the *AURKA* gene in HNSC was analyzed using the UALCAN database. Expression in different grades of the tumor was assessed viz., grade 1 – well-differentiated, grade 2 - moderately differentiated, grade 3 - poorly differentiated, grade 4 - undifferentiated. Transcripts per million (TPM) is a normalization method for RNA-seq data. The TPM values used for the generation of box-whisker plots were also used to determine the significant difference between the groups. The t-test was performed using a PERL script with the Comprehensive Perl Archive Network (CPAN) module. Combined survival effect analysis of gene expression and other clinical parameters such as race, gender, tumor grade, cancer subtypes were assessed using multivariate Kaplan-Meier survival analysis.¹²

RESULTS AND DISCUSSION

AURKA gene overexpression has been demonstrated in various human cancers and Treekitkarnmongkol et al, state that Aurora kinase-A may be an important genetic event in cancer development.^{13,14} The abnormal expression of the *AURKA* gene might lead to high chromosomal instability in tumors and further increase susceptibility to malignant transformation.¹⁵ The overexpression promotes cell proliferation, tumor progression, and metastasis.¹⁶ The blocking of *AURKA* induces apoptosis and autophagy in mice and significantly increases sensitivity to chemical treatment in oral squamous cell carcinoma.¹⁷ These findings provide an insight that *AURKA* breakdown might be a valuable therapeutic strategy for

oral squamous cell carcinoma.

The present study compared the genotype frequency data acquired from the Ensembl database for *rs2064863* polymorphism which was used for further analysis. The global allele frequency for T alleles and G alleles were found to be 63% and 37%, respectively (Figure 1). The allele frequencies documented in different populations have been depicted in Figure 2. To have a clearer picture of the deviations observed in allele frequencies between different populations, a comparative analysis was carried out using the data presented by Chou et al, in the Taiwanese population (East Asian). The comparison of allele frequency between global data [T allele (63%) - G allele (37%)] with case [T allele (81.7%)-G allele (18.3%)] and control group data [T allele (83%)-G allele (17%)] are represented in Figure 3a and 3b respectively. Comparison of allele frequency was also made between East Asian data from the database [T allele (81%)-G allele (19%)] with the case [T allele (81.7%)-G allele (18.3%)] and control group [T allele (83%)-G allele (17%)] from the genetic analysis by Chou et al. which is represented in Figure 4a and 4b. Previous studies demonstrated *AURKA rs2064863* polymorphism was associated with a high risk of stage three and stage for oral squamous cell carcinoma but not with tumor size, metastasis to lymph nodes and distant organs, cell differentiation.¹⁸

The expression profile of *AURKA* gene in HNSCC patients using *in silico* tools demonstrated that the expression was found to increase with the grade indicating that *AURKA* might have a role to play in tumor progression (Figure 5). Even though *AURKA* gene expression did not vary between different ethnic groups, there was a significant difference between the *AURKA* expression between normal subjects and different races (data not shown). This result agreed with several studies that report on the up-regulation of *AURKA* gene during tumor state. The *AURKA* gene expression based on different grades of the tumor is represented in Figure 5, of which several groups showed significant differences. In addition, the Kaplan-Meier method was used to assess the survival probability of HNSCC patients based on the expression of the selected gene. The results showed a significant difference in the survival rate of patients with high and medium/low-level expression of *AURKA* gene [$p = 0.038$]. Increased expression correlated with a poor survival probability of HNSC patients (Figure 6a). Also, high expression of *AURKA* gene correlated with low survival rate in African-American patients when compared to low/medium level expression in Caucasians (Figure 6b). This kind of observation may be attributed to the cumulative effect of variants that are positively selected in a population.

AURKA gene overexpression was strongly associated with the progression of colorectal adenoma to colorectal cancer. Hardebol et al. also observed a similar kind

of results employing *in vitro* studies.¹⁹ However, the possibility of an association among the advanced stage, expression, and *AURKA* genotype as well as the effects of the *AURKA* genotype on oral cancer risk, require further investigation. *In silico* studies have gained a lot of focus during recent times due to their enormous application in medical and clinical research. An exhaustive collection of data can be analyzed within a short period to provide preliminary evidence on the study design which can be further validated using experimental approaches.^{20, 21} Photographs of the patients aid in recording the clinical data of oral cavity.^{22, 23} Understanding the role of immunohistochemical biomarkers like cyclin D1, p27, and p63 will provide an insight regarding the transformation of potentially malignant disorder into malignant disorders.^{24, 25} Immunohistochemical study of giant cells present in tumors and tumor-like lesions gives a better understanding of disease development.^{26, 27} Mouse cytomegalovirus induces mucoepidermoid carcinoma and further study will provide insight regarding the relationship between them in humans.²⁸

Aurora A has an oncogenic role in breast cancer development. Over-expression of Aurora A is also seen in non-malignant tissues, which might help in the early detection of breast cancer.²⁹ In primary ovarian tumors, increased levels of *AURKA* are associated with supernumerary centrosomes, which decrease the survival rate of the patients, signifying the role of *AURKA* in ovarian cancer biology.³⁰ *AURKA* interacts with oncogenic signal pathways and suppresses the tumor suppressor functions of p53 and p27 in cancer cells. It also regulates carcinogenic properties like angiogenesis, invasion, inflammation, and cell survival which were observed in gastrointestinal cancer.³¹ *AURKA* overexpression was also observed in esophageal squamous cell carcinoma.³² Non-small cell lung cancers (NSCLC) demonstrated *AURKA* expression predominantly in certain subtypes of NSCLC.³³ *AURKA* has functional diversity making it an excellent target in cancer therapy. It has specific inhibitors that significantly reverse tumor growth.³⁴ The present study showed that there was a significant deviation in the allele frequency of rs2064863 polymorphism of *AURKA* gene which might be positively selected in the South Asian population. This positive selection could have implications in the tumor development and progression which might be related to exposure to environmental carcinogens or habits.

CONCLUSION

To conclude, experimental validation and genotyping in the south Indian population would be the best strategy to derive an association between oral cancer and *AURKA* gene polymorphism, since the significant difference in the survival rate of patients with high and medium/low-level expression

of *AURKA* gene correlated with the survival probability of the patients. The results could also help in drawing conclusions about the positive selection of alleles in the South Indian population, which might have an implication in the progression of the tumor in head and neck cancer patients.

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Figure 1: Pie chart depicting global allele frequency data for the rs2064863 polymorphism of AURKA gene as acquired from the Ensembl database (T allele frequency = 63%; G allele frequency = 37%).

Figure 2: Bar chart depicting allele frequency data for the rs2064863 polymorphism of AURKA gene among different populations. X-axis denotes different populations. Y-axis denotes the percentage of T and G allele frequency in different populations.

Figure 3a: Bar chart depicting comparison of allele frequency of case from the study carried out by Chou et al. with global population of *rs2064863* polymorphisms of *AURKA* gene. X-axis denotes the global and case population. Y-axis denotes the percentage of T and G allele frequency in the global and case population.

Figure 3b: Bar chart depicting comparison of allele frequency of control from the study carried out by Chou et al. with global population of *rs2064863* polymorphisms of *AURKA* gene. X-axis denotes the global and control population. Y-axis denotes the percentage of T and G allele frequency in the global and control population.

Figure 4a: Comparison of allele frequency of case from the study carried out by Chou et al. with East Asian population of *rs2064863* polymorphisms of *AURKA* gene. X-axis denotes the East Asian and case population. Y-axis denotes the percentage of T and G allele frequency in East Asian and case population

Figure 4b: Bar chart depicting comparison of allele frequency of control from the study carried out by Chou, et al with East Asian population of *rs2064863* polymorphisms of *AURKA* gene. X-axis denotes the East Asian and control population. Y-axis denotes the percentage of T and G allele frequency in East Asian and control population.

Figure 5: Box-whisker plot showing relative expression of *AURKA* in normal and different grades of tumor in HNSC patients. The X-axis denotes grades of tumor relative to normal expression of *AURKA* and Y-axis denotes mRNA counts expressed as TPM (transcripts per million). The comparison of gene expression pattern between different grades of HNSC returned significant values between Normal vs. Grade 1 ($p=1.6 \times 10^{-12}$), Normal vs. Grade 2 ($p=1.6 \times 10^{-12}$), Normal vs. Grade 3 ($p= <1 \times 10^{-12}$), Normal vs. Grade 4 ($p=3.7 \times 10^{-5}$), Grade 1 vs. Grade 2 ($p=1.8 \times 10^{-5}$), Grade 1 vs. Grade 3 ($p=5.2 \times 10^{-9}$), Grade 1 vs. Grade 4 ($p=2.2 \times 10^{-4}$), Grade 2 vs. Grade 3 ($p=2.2 \times 10^{-3}$).

Figure 6: Kaplan–Meier plots showing the association of *AURKA* expression and other clinical parameters with patient survival. The X-axis represents time in days and Y-axis shows the probability of surviving (a) KM plot depicting the effect of differential *AURKA* gene expression on the survival of HNSC patients with different grades of tumor (p value = 0.038) (b) KM plot depicting the effect of *AURKA* gene expression level and race (high level expression in African-American vs. low/medium level expression Caucasian population, p value = 0.013) on HNSC patient's survival.