Name: Nisreen Sherif Alyasiri

Department of Biosciences

Supervisor: Dr. Moshahid Alam Rizvi

Co-supervisors: Dr.A.K.Mandal; Dr. Sunita Gupta

Title: Assessment on the role of PTEN tumor suppressor gene in oral cancer.

Abstract

Oral cancer is the sixth most common cancer world wide. In India oral cancer is the commonest cancer accounting for 50-70% of total cancer mortality. As the incidence of oral cancer continues to increase, the disease becomes an increasingly important public health issue. Oral cancer sites are readily amenable to clinical examination, yet a lack of suitable molecular marker is distinctly reflected by the fact that more than 50% of all oral squamous cell carcinoma patients have advanced disease at the time of diagnosis. This underlines the importance of the present study which paves the way to possible molecular markers. PTEN gene is one of the most frequently mutated Tumor Suppressor Genes in human cancers. Studies on PTEN in OSCC showed frequent genetic alterations and loss of expression but still there are a lot of discrepancies toward the drawing of the final conclusion.

In India, this is probably the first study conducted to investigate the role of PTEN gene in oral cancer. The present study is an attempt to explore PTEN gene at protein expression/apoptotic level, and genetic/epigenetic level. Further, a survey study was conducted to explore the effect of some epidemiological factors and their relevance with oral cancer in Indian population.

The present study includes 146 formalin fixed tissue sections of oral squamous cell carcinoma obtained from department of pathology Maulana Azad Medical College- New Delhi. Further, one hundred fresh incisional oral tumor biopsies and their matched control blood samples were collected from Lok Nayak Jai Parakash associated hospital- Maulana Azad medical college – New Delhi. PTEN expression was explored by immunohistochemistry analysis. Loss of PTEN expression occurred in 61% of the studied cases. A significant correlation was observed between PTEN expression and tumor stage (*p* value=0.002) suggesting that tumors with low level of PTEN expression may show more aggressiveness. Apoptotic phenomenon was also determined through (TUNEL) assay

following the manufacture's protocol. High apoptotic index was observed in 105 (72%) of samples, and it was found to be significantly related with loss of PTEN expression (*p* value=0.02). The present study confirms the contribution of loss of PTEN expression in spontaneous apoptosis suppression in the specimens of oral cancer. All patients were interviewed uniformly. The questionnaire used in the interview sought detailed information on current and past cigarette smoking, betel chewing, alcohol drinking and dietary habits. The present study showed that 65% of patients were smokers, 48% were consuming smokeless tobacco, 24 % were consuming alcohol. The habitual smokers and chewers showed typical bias towards the pathogenesis. Our findings add to the existing body of evidence which suggest smoking, tobacco chewing and drinking alcohol as the highly significant risk factors for oral cancer. The risk of oral cancer seems to increase in non-vegetarian patients and patients who do not take fruits frequently. Mutational analysis was conducted by examining the entire exons of the gene by PCR-based single stranded conformational polymorphism (PCR-SSCP). No mobility shift was observed referring that there was no any mutation. Some other investigators have also suggested no relationship between PTEN genetic alteration and oral carcinoma existed. We agree to state the same but only at mutational level, apoptotic/expression data suggest otherwise. Using immunohistochemical analysis of OSCC from 146 cases worth to demonstrate that the rate of inactivation of PTEN gene at the protein level is more frequent than that at the genetic level. For DNA Methylation analysis, Genomic DNA specimens were subjected to bisulfite treatment using EZ DNA Methylation-Gold ™ kit (Zymo Research, USA). PTEN promoter hypermethylation occurred in 35% of the studied cases and found to be significantly correlated with tumor grade (*p* value=0.0019). Our finding concludes the existence of abnormal methylation of PTEN gene promoter in the oral squamous cell carcinoma, test of PTEN promoter hypermethylation can be taken as promising strategy to be employed in the diagnosis of OSCC. No such study so far has been made among OSCC from Indian population. We for the first time reporting the level of PTEN methylation in OSSC among Indian population. We further suggest that additional studies are required to elucidate the role of PTEN gene in this cancer type.