uicc
global cancer control

IJC
International Journal of Cancer

Promoter hypermethylation in Indian primary oral squamous cell carcinoma

Jatinder Kaur[1], Semra Demokan[2], Satyendra Chandra Tripathi[1], Muzafar Ahmad Macha[1], Shahnaz Begum[3], Joseph A. Califano[2] and Ranju Ralhan[1,4,5,6,7,8,9]

[1] Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India
[2] Department of Otolaryngology-Head and Neck Surgery, John Hopkins Medical Institutions, Baltimore, MD
[3] Department of Pathology, John Hopkins Medical Institutions, Baltimore, MD
[4] J & M Sonshine Family Centre for Head and Neck Diseases, Mount Sinai Hospital, Toronto, ON, Canada
[5] Department of Otolaryngology-Head and Neck Surgery, Mount Sinai Hospital, Toronto, ON, Canada
[6] Alex and Simona Shnaider Research Laboratory in Molecular Oncology, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada
[7] Department of Otolaryngology-Head and Neck Surgery, University of Toronto; Toronto, ON, Canada
[8] Department of Chemistry, York University, Toronto, ON, Canada
[9] Centre for Research in Mass Spectrometry, York University, Toronto, ON, Canada

We evaluated promoter hypermethylation of a panel of tumor suppressor genes as a means to detect epigenetic alterations in oral squamous cell carcinomas (OSCC) of Indian-origin and compare with North-American head and neck squamous cell carcinomas (HNSCC). Quantitative-methylation-specific PCR was used to investigate the promoter methylation status of *DCC*, *EDNRB*, $p16^{INK4a}$ and *KIF1A* in 92 OSCC, and compared to 48 paired normal tissues and 30 saliva and sera samples from healthy control subjects. Aberrant methylation of at-least one of these genes was detected in 74/92 (80.4%) OSCC; 72.8% at *EDNRB*, 71.7% at *KIF1A*, 47.8% at $p16^{INK4a}$ and 58.7% at *DCC*; and in 5 of 48 (10.4%) normal oral tissues. None of the saliva and sera samples from controls exhibited DNA methylation in these four target genes. Thirty-two of 72 node positive cases harbored $p16^{INK4a}$ and *DCC* hypermethylation ($p = 0.005$). Thus, promoter hypermethylation in genes analyzed herein is a common event in Indian OSCC and may represent promising markers for the molecular staging of OSCC patients. We found higher frequency of $p16^{INK4a}$ methylation (47.8%) in this Indian cohort in comparison with a North-American cohort (37.5%). In conclusion, aberrant methylation of *EDNRB*, *KIF1A*, *DCC* and $p16^{INK4a}$ genes is a common event in Indian OSCC, suggesting that epigenetic alterations of these genes warrant validation in larger studies for their potential use as biomarkers.

Head and neck squamous-cell carcinoma (HNSCC) is the sixth most common cancer in United States and the fourth most prevalent cancer in men worldwide.[1] Rapid advances in treatment modalities and improvements in the early detection of HNSCC have not significantly impacted the overall survival rates of cancer patients (about 50% at 5 years). Development of novel biomarkers offer the potential to transform clinical practice by improving the efficacy of cancer screening and diagnosis based on molecular markers as a complement to routine clinical screening and diagnostic strategies. An epigenetic pathway of transcriptional inactivation for many tumor suppressor genes includes CpG island hypermethylation within promoter regions.[2–5] This pathway has been identified in several human cancers including HNSCC.[5–8] Promoter hypermethylation is a powerful and ubiquitous mechanism of gene silencing which can be detected in tissue samples using quantitative methylation-specific PCR (Q-MSP); this real-time PCR methodology enables an objective, robust, and

Key words: hypermethylation, EDNRB, KIF1A, OSCC, $p16^{INK4a}$, DCC, nodal metastasis

Abbreviations:: CDK: cyclin dependent kinase; DCC: deleted in colorectal carcinoma; EDNRB: endothelin receptor type B; ET: endothelin; HNSCC: head and neck squamous-cell carcinoma; KIF1A: kinesin chain member 1A; OSCC: oral squamous cell carcinoma; Q-MSP: quantitative methylation-specific PCR

Grant sponsors: Department of Biotechnology, India, Joseph and Mildred Sonshine Family Centre for Head and Neck Diseases, Mount Sinai Hospital and Temmy Latner/Dynacare Family Foundation, Toronto, Canada
DOI: 10.1002/ijc.25377
History: Received 28 Sep 2009; Accepted 12 Feb 2010; Online 5 Apr 2010

Correspondence to: Joseph A. Califano, Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins Medical Institutions, 601 North Caroline Street, 6th Floor, Baltimore, MD 21287-0910, Tel: 410-502-5133, Fax: 1-410-614-1411, E-mail: jcalifa@jhmi.eduor Milton J. Dance Head and Neck Center, Greater Baltimore Medical Center, Baltimore, MD; or Ranju Ralhan, Joseph and Mildred Sonshine Family Centre for Head and Neck Diseases, Mount Sinai Hospital, Joseph & Wolf Lebovic Health Complex, 600 University Avenue, Room 6-500, Toronto, Ontario, Canada M5G 1X5, Tel: 1-416-586-4800 x 6426, Fax: 1-416-586-8628, E-mail: rralhan@mtsinai.on.ca