GENOME-WIDE ANALYSIS OF GENETIC ALTERATIONS IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA BY SNP ARRAY

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Introduction and Background: Cancer is the result of a series of genetic or epigenetic changes, including aneuploidy, multiple gene amplification, deletions and translocations. These genetic instabilities are caused by either inherited mutations in genes that monitor genome integrity or mutations that are acquired in somatic cells during tumor development. Environmental risk factors and individual cancer genetic susceptibilities could contribute to tumor development and progression by facilitating the inactivation or loss of tumor suppressor genes and by favoring the activation or amplification of oncogenes. The development of esophageal cancer is a leading example in which environmental carcinogens in addition to geographic and genetic factors appear to play major etiologic roles. Esophageal cancer occurs at very high frequencies in certain parts of China, Iran, South Africa, Uruguay, France, Italy and in some regions of India. The highest incidence of this cancer in India has been reported from Assam (AAR of 33/100,000) in the North-east region where it is the second leading cancer in men and third leading cancer in women. Tobacco smoking, betel quid chewing, and alcohol consumption are the major known risk factors for esophageal cancer. Fermented areca nut chewing with or without tobacco has been shown to be independently associated with the development of esophageal squamous cell carcinoma in Assam region of NE India. However, genome-wide analysis of genetic alterations of esophageal cancer in this high incidence region of India has so far not been investigated.

Purpose: In the current study, samples obtained from germ line and tumor DNA were analyzed to establish a high-resolution chromosomal instability profile using GeneChip Human Mapping 10K Array Xba 142 2.0. The aim was if some common chromosomal aberrations (deletion or amplification) are present in patients with ESCC from a high-incidence region of India.

Methods: Germ-line DNA and tumor tissue DNA was extracted using the Qiagen QIamp DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instruction. Further sample processing, including digestion, adaptor ligation, amplification, fragmentation, labeling, hybridization , washing and scanning was assayed according to the standard protocol (Affymetrix GeneChip Mapping 10K 2.0 Assay Manual). CEL files, containing intensity value and standard deviation for each probe on the chip were generated for each array using the GeneChip Operating Software (Affymetrix). Perfect match-mismatch average difference intensities as a signal to noise value and the genotypes (AA, AB and BB) were calculated for each biallelic SNP using the GeneChip DNA Analysis Software (GDAS) and GTYPE v4.0 (Affymetrix). For further data analysis and illustration of the results, the CEL files were imported into the Copy Number Analysis Tool 4.0 software (CNAT) from Affymetrix. Quantile normalization was performed for all the arrays. Log2 ratio values were imported from CNAT to DNA Analytics 4.0 CGH Module (Agilent Technologies) to find out the common chromosomal aberration in the sample.

Results: Tumor tissue and matched peripheral blood obtained from twenty patients with ESCC were analyzed for allelic imbalance and copy number alterations. The average genotyping call rates for the tumor and matched

normal samples were 95.54 % and 95.63% respectively. Frequent amplifications were found on chromosomes arms 1p36.13, 1q21.1, 2p14, 3q28, 3q27, 3q26.1, 5p15.2, 5q11.2, 6p25.3, 7q11.21, 9q31.3, and17p13.1. Frequent gain abnormalities were found on chromosome arms 1q, 3q, 5p, 7p, 8q, 11q, 12p, and 12q. Frequent deletions were found on chromosome arms 1p, 3p, 4p, 5q, 8p, 9p, 11q, 13q, 17p, and 18q.

Conclusion: Some of the chromosomal regions that showed gain or losses contain cancer genes known to be involved in ESCC and others hold genes that have a known role in other cancers but which have yet to be established as esophageal cancer genes. Our analysis revealed a characteristic pattern of genomic imbalances in squamous cell carcinoma tissue from patients with esophageal cancer and identified genomic regions that are strongly associated with tumor initiation, metastasis and high-grade disease.In addition these regions may contain ESCC-related genes and provide important theoretic information for identifying and cloning novel ESCC-related oncogenes and tumor suppressor genes.