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BASIC AND TRANSLATIONAL—ALIMENTARY TRACT

Genomic Landscape of Esophageal Squamous Cell Carcinoma in a Japanese Population

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BACKGROUND & AIMS: Esophageal squamous cell carcinoma (ESCC) is the predominant form of esophageal cancer in Japan. Smoking and drinking alcohol are environmental risk factors for ESCC, whereas single nucleotide polymorphisms in *ADH1B* and *ALDH2*, which increase harmful intermediates produced by drinking alcohol, are genetic risk factors. We conducted a large-scale genomic analysis of ESCCs from patients in Japan to determine the mutational landscape of this cancer. **METHODS:** We performed whole-exome sequence analysis of tumor and non-tumor esophageal tissues collected from 144 patients with ESCC who underwent surgery at 5 hospitals in Japan. We also performed single-nucleotide polymorphism array-based copy number profile and germline genotype analyses of polymorphisms in *ADH1B* and *ALDH2*. Polymorphisms in *CYP2A6,* which increase harmful effects of smoking, were analyzed. Functions of *TET2* mutants were evaluated in KYSE410 and HEK293FT cells. **RESULTS:** A high proportion of mutations in the 144 tumor samples were C to T substitution in CpG dinucleotides (called the CpG signature) and C to G/T substitutions with a flanking 5′ thymine (called the APOBEC signature). Based on mutational signatures, patients were assigned to 3 groups, which associated with environmental (drinking and smoking) and genetic (polymorphisms in *ALDH2* and *CYP2A6*) factors. Many tumors contained mutations in genes that regulate the cell cycle (*TP53, CCND1, CDKN2A, FBXW7*); epigenetic processes (*MLL2, EP300, CREBBP, TET2*); and the NOTCH (*NOTCH1*, *NOTCH3*), WNT (*FAT1*, *YAP1*, *AJUBA*) and receptor-tyrosine kinase−phosphoinositide 3-kinase signaling pathways (*PIK3CA*, *EGFR*, *ERBB2*). Mutations in *EP300* and *TET2* correlated with shorter survival times, and mutations in *ZNF750* associated with an increased number of mutations of the APOBEC signature. Expression of mutant forms of *TET2* did not increase cellular levels of 5-hydroxymethylcytosine in HEK293FT cells, whereas knockdown of *TET2* increased the invasive activity of KYSE410 ESCC cells. Computational analyses associated the mutations in *NFE2L2* we identified with transcriptional activation of its target genes. **CONCLUSIONS:** We associated environmental and genetic factors with base substitution patterns of somatic mutations and provide a registry of genes and pathways that are disrupted in ESCCs. These findings might be used to design specific treatments for patients with esophageal squamous cancers.

Keywords: Mutational Signature; Exome Sequencing; Copy Number Profiling; Esophagus.

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive types of cancer, frequently showing lymph node metastasis and tumor invasion into adjacent organs, even in the early stages.[1,2] In contrast to the predominance of adenocarcinoma in Western countries, squamous cell histology represents the most prevalent form of esophageal cancer in East Asia, including Japan and China.

In Japan, ESCC is the tenth most common malignancy and the seventh most common cause of cancer-related deaths. Epidemiologic studies have established that drinking and smoking are strong risk factors for developing ESCC.[3] Furthermore, genetic polymorphisms that impair the functions of alcohol-metabolizing enzymes have been reported as risk factors. Our previous genome-wide association studies have shown that functional single-nucleotide polymorphisms (SNPs) in 2 alcohol dehydrogenase genes, *ADH1B* (rs1229984 GG) and *ALDH2* (rs671 AG/AA), increase the incidence of ESCC in Japanese cohorts.[4] These findings are consistent with data from another independent study.[5] Notably, there is drastic synergy among these genetic and environmental risk factors; our study showed that the incidence odds ratio was as high as 146.4 (95% confidence interval [CI]: 50.5−424.5) in the presence of all 4 risk factors.

In China, the incidence and mortality rates of ESCC are higher than those in Japan. ESCC is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer-related deaths in China. There are substantial differences in the epidemiology[3] of ESCC between the Japanese and Chinese populations. The association of smoking and drinking with the risk of developing ESCC appears to be weaker in China than in Japan. Notably, a regional bias exists in the incidence of Chinese ESCC. Although the incidence rate is generally higher in rural areas than in urban areas throughout China, some rural areas have reported particularly high incidences. In the high-incidence areas, the association between the incidence rate and drinking and smoking behavior is especially weak; instead, family history and environmental factors, such as nutritional deficiency and food mutagens, are proposed risk factors.[6–8]

Recently, several whole-exome sequencing (WES) studies have revealed different landscapes of driver genes, as well as somatically disrupted pathways in ESCC.[9,10] Notably, 3 large-scale WES studies of ESCC cohorts have been carried out in China; Gao et al[11] evaluated a low-incidence cohort from an urban area near Beijing, while Song et al[12] and Zhang et al[13] examined high-incidence cohorts from the Chaoshan District of Guangdong Province and the Taihang Mountains of north central China, respectively. However, no comprehensive genomic profiling of a Japanese ESCC cohort has been reported.

Here, we present the landscape of genomic alterations in Japanese ESCC patients obtained by WES and/or SNP array-based copy number (CN) analysis in 144 Japanese patients. This study not only expands the registry of somatically disrupted driver genes, but also reveals the association of genotype−environment interactions with mutational signatures that are unique to the Japanese ESCC cohort.

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Material and Methods

Sample Collection

Samples from 144 patients with diagnosed ESCC were obtained from 5 hospitals (Juntendo University Hospital, National Cancer Center Hospital, Kurume University Hospital, Saitama Cancer Center, and Kagoshima University Hospital). Written informed consent was obtained from all patients and the study protocol was reviewed and approved by the internal review board of Kyushu University.

Whole-Exome Sequencing

DNA extracted from ESCC and paired normal samples was captured using the SureSelect Human All Exon 50Mb Kit (Agilent Technologies, Santa Clara, CA) following the manufacturer's instructions. Captured DNA was sequenced using the HiSeq2000 and paired-end (75−100 bp) sequencing reads were generated for each sample. Mutation calling was performed using the EBcall algorithm[14] (http://genomon.hgc.jp/exome/en/index.html).

Copy Number Profiling

DNA was processed and hybridized to the HumanOmniExpress BeadChip (HumanOmniExpress BeadChip Kit; Illumina, San Diego, CA) following the manufacturer's protocol. GenomeStudio (Illumina) was used to obtain B-allele frequencies and normalized logarithmic probe intensities, which were used as input data for an ASCAT analysis to estimate CN profiles along with tumor ploidy and aberrant cell fraction.[15] For details, please see the Supplementary Material and Methods.

Results

Effects of Environmental/Genetic Factors on Mutational Signatures

Tumor and paired normal DNA from 144 Japanese ESCC patients were subjected to WES. The mean read depth was 120× and 91.1% of target bases were covered by >10 independent reads (Supplementary Tables 1 and 2). A total of 23,121 somatic events, including 22,175 single-nucleotide substitutions and 946 short insertions and deletions (indels), were identified. The mean number of mutations was 161 (range, 47−644) per sample or 3.10 (range, 0.91−12.4) per megabase across the target exome sequences (Figure 2*A* and Supplementary Table 3). Similar to previous findings for many cancer types, the predominant substitution across all ESCC samples was C to T involving the CpG dinucleotide (Figure 1*A*).[16] In addition, C to

***Authors share co-first authorship.**

Abbreviations used in this paper: **CI, confidence interval; CN, copy number; ESCC, esophageal squamous cell carcinoma; HNSCC, head and neck squamous cell carcinoma; PI3K, phosphoinositide 3-kinase; RTK, receptor-tyrosine kinase; SNP, single-nucleotide polymorphism; WES, whole-exome sequencing.**

Most current article

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