ORIGINAL ARTICLE

Validation of *SCT* Methylation as a Hallmark Biomarker for Lung Cancers

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ABSTRACT

Introduction: The human secretin gene (*SCT*) encodes secretin, a hormone with limited tissue distribution. Analysis of the 450k methylation array data in The Cancer Genome Atlas (TCGA) indicated that the *SCT* promoter region is differentially hypermethylated in lung cancer. Our purpose was to validate *SCT* methylation as a potential biomarker for lung cancer.

Methods: We analyzed data from TCGA and developed and applied *SCT*-specific bisulfite DNA sequencing and quantitative methylation-specific polymerase chain reaction assays.

Results: The analyses of TCGA 450K data for 801 samples showed that *SCT* hypermethylation has an area under the curve (AUC) value greater than 0.98 that can be used to distinguish lung adenocarcinomas or squamous cell carcinomas from nonmalignant lung tissue. Bisulfite sequencing of lung cancer cell lines and normal blood cells allowed us to confirm that *SCT* methylation is highly discriminative. By applying a quantitative methylation-specific polymerase chain reaction assay, we found that *SCT* hypermethylation is frequently detected in all major subtypes of malignant non–small cell lung cancer (AUC = 0.92, n = 108) and small cell lung cancer (AUC = 0.93, n = 40) but is less frequent in lung carcinoids (AUC = 0.54, n = 20). *SCT* hypermethylation

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Drs. Zhang, Ma, and Sathe contributed equally to this work.

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appeared in samples of lung carcinoma in situ during multistage pathogenesis and increased in invasive samples. Further analyses of TCGA 450k data showed that *SCT* hypermethylation is highly discriminative in most other types of malignant tumors but less frequent in low-grade malignant tumors. The only normal tissue with a high level of methylation was the placenta.

Conclusions: Our findings demonstrated that *SCT* methylation is a highly discriminative biomarker for lung and other malignant tumors, is less frequent in low-grade malignant tumors (including lung carcinoids), and appears at the carcinoma in situ stage.

Keywords: SCT methylation; Cancer biomarker; Lung cancer; FFPE DNA SCT qMSP; Secretin

Introduction

Lung cancer is the leading cause of cancer-related death in the United States and worldwide and is estimated to have caused more than 1.3 million deaths in 2011.[1,2] There remains an urgent need for specific and sensitive molecular biomarkers for lung cancer that can be applied in development of cost-effective tests to facilitate diagnosis of lung cancer at an early stage and assess risk.

The methylation of cytosine adjacent to guanosine in the cytosine-phosphate-guanine (CpG) island sequence in the promoter region may lead to silencing of expression of genes, including tumor suppressor genes, in lung and other cancers.[3,4] Genome-wide analysis of DNA methylation has shown altered DNA methylation between lung tumor and nonmalignant lung tissue that affects expression of multiple genes with an important role in carcinogenesis.[5,6] Of interest, the onset and level of aberrant changes in epigenetic DNA methylation commence during the multistage pathogenesis of lung cancer.[7] Aberrant DNA methylation may be useful as a biomarker for early diagnosis, prognosis, and risk assessment, as well as for management of therapy for patients with cancer.[8–10] The many promising DNA methylation biomarkers for lung cancer that have been discovered include the cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*), telomerase reverse transcriptase gene (*TERT*), Ras association domain–containing protein 1 gene (*RASSF1A*), *O*-6-methylguanine-DNA methyltransferase gene (*MGMT*), and short stature homeobox 2 gene (*SHOX2*).[11–17]

The human secretin gene (*SCT*) is located on chromosome 11p15.5 and encodes the precursor gene of secretin, an endocrine hormone. Secretin, a 27-amino acid peptide, was the first hormone discovered. It was discovered in 1902 by Bayliss and Starling, and their finding initiated the field of endocrinology.[18,19] The major site of production of secretin is the endocrine S cells located in the proximal small intestinal mucosa.[20] Secretin is secreted by the gut in response to food containing fatty acids or having an acidic pH.[21] Secretin stimulates the secretion of bicarbonate-rich pancreatic fluids and is also associated with release of bile from the liver, release of gastric pepsin from the stomach, and inhibition of gastric acid.[22] Human secretin transcripts have a limited distribution in normal tissue, including the intestinal tract, spleen, and brain tissue.[23] Secretin may perform neuroendocrine functions in addition to playing a role in digestion, and it may be associated with autism.[21,22] Expression of the *SCT* gene may be regulated through the combined effect of the ratio of the trans-acting factors Sp1 and Sp3 through GC boxes, the enhancer box element, and CpG DNA methylation status in the *SCT* promoter region.[24] Treatment with the demethylation agent 5′-aza-2′-deoxycytidine induced substancial expression of secretin in the *SCT* fully methylated human pancreatic and hepatocellular carcinoma cell lines.[24] Secretin inhibited the growth of human cholangiocarcinoma cell lines and tumor mouse xenografts, thus implying that it has tumor suppressor–like effects on tumor cells.[25]

From analysis of the methylation 450k array data in The Cancer Genome Atlas (TCGA), X. M. and M. Q. Z. observed that the promoter region of the *SCT* gene is selectively hypermethylated in non–small cell lung cancer (NSCLC). The purpose of this study was to extend this finding and validate *SCT* methylation as a potential biomarker for lung and other cancers.

Materials and Methods

Analyses of 450k Methylation Array Data

The Illumina HumanMethylation450 BeadChip (Illumina, San Diego, CA) was used to profile the methylation status of primary lung tumors and nonmalignant tissue samples in the data sets of TCGA as well as that of 147 lung cancer cell lines from our laboratory (A.F.G.) (see Supplementary Table 1 in Supplemental Digital Content 1). The 450k methylation array data in TCGA regarding the *SCT* gene for the primary tumors that were studied in this report and the data on variation of lung tumor copy numbers were obtained from the data portal of TCGA[26] (see Supplementary Tables 2–5 in Supplemental Digital Content 1 for complete list of samples and data). Eleven cases in the 450k methylation data sets for lung adenocarcinoma in TCGA were found to have multiple entries from different samples examined; their data ($n = 26$) were excluded from our receiver operator characteristics (ROC) curve analysis to avoid any bias from