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Original contribution

Genomic Copy Number Signatures Uncovered a Genetically Distinct Group from Adenocarcinoma and Squamous Cell Carcinoma in Non–Small Cell Lung Cancer☆,☆☆

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Summary Adenocarcinoma (AC) and squamous cell carcinoma (SCC) of non–small cell lung carcinoma (NSCLC) have different clinical presentations, morphologies, treatments, and prognoses. Recent studies suggested that fundamental genetic alterations related to carcinogenesis of each tumor type may be different. In this study, we investigated the genomic alterations of 47 primary NSCLC samples (22 ACs and 25 SCCs) as well as the corresponding normal tissue using array comparative genomic hybridization. Frequent copy number alterations (CNAs), which were identified in more than 68% of all of the cases, were evaluated in each subtype (SCC and AC), and a CNA signature was established. Among these CNAs, 37 genes from the SCCs and 15 genes from the ACs were located in a region of gain, and 4 genes from the SCCs and 13 genes from the ACs were located in a region of loss. The most frequent gain was located on 3q26-29 including the gene *TP63* in SCCs and 7q11.23 and 7q36.3 in ACs. Moreover, we identified 3 genetically distinct groups (group I [16 SCC] with CNA signature of SCC; group II [7 SCC + 8 AC], which has a genetically distinctive CNA signature from SCC and AC; and group III [2 SCC + 14 AC] with CNA signature of AC) by gene clustering extracted from CNAs, which are associated with a prognosis. The present study contributed to the molecular characterization of AC and SCC of NSCLC and showed a subtype of tumor that has a unique genetic CNA signature. However, further study about the significance of these 3 distinct groups and their usefulness as a diagnostic marker of identified CNAs is necessary.

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1. Introduction

Lung cancer remains a leading cause of mortality in cancer worldwide, and its incidence has been increasing in Asia [1]. Lung cancer is treatable with curative intent by complete surgical resection in early stage tumors only. Unfortunately, most patients are diagnosed at the advanced, inoperable stages of the disease, which is often associated with tumor spread to regional lymph nodes and distant sites. Despite the technical improvements of lung cancer therapy that have been achieved in the past few decades, the overall 5-year survival rate after diagnosis remains less than 16% [2]. Therefore, the identification of treatments that use target molecules, which were identified using highly advanced or technologically advanced molecular analyses, has been highlighted in the cancer research field. Over the last decade, molecular profiling of a vast number of human tumors has generated catalogues of genomic alterations for specific cancer types resulting in a better understanding of cancer biology and also in the development of targets for rational therapies. This has already led to several targeted therapy regimens, such as inhibition of the *EGFR* and *VEGFR* as well as the *EML4-ALK* gene fusion [3]. Although several genetic mutations have been reported previously, a large proportion of patients with lung cancer do not exhibit these mutations in their cancer genome. More than 40% of lung cancers appear to be driven by unknown genetic events [4,5]. Histologically different lung cancers, particularly squamous cell carcinoma (SCC) and adenocarcinoma (AC) of non–small cell lung carcinoma (NSCLC), have different genetic changes, and even with the same genetic changes, response to a treatment is quite different [6]. Currently, epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitor therapy is given to patients who have AC of the lung with an *EGFR* mutation. It is less effective, however, in *EGFR*-mutated SCC cases of the lung [7]. Therefore, it is very important to distinguish AC and SCC of NSCLC to determine the appropriate treatment protocol and prognostic value. Most of the computer tomography–guided lung biopsy specimens are easy to distinguish SCC from AC morphologically or immunohistochemically. However, in some cases, such as AC with a squamoid solid pattern and no expression of thyroid transcription factor-1 or napsin, differential diagnosis between SCC and AC by histology could be difficult and genetic information may help determine the correct diagnosis [8].

Genomic DNA copy number alterations (CNAs) in various cancers have been shown to be associated with cancer development and progression [9]. If CNAs are involved in the amplification of proto-oncogenes that regulate cell division or in the loss of tumor suppressor genes that prevent unwanted cell division or induce programmed cell death, these errors can be contributing factors in the initiation stage of carcinogenesis. Accumulation of particular combinations of these genetic errors can cause a group of cells to cross the threshold to cancer. At that point, the cells' increased genetic instability and high replication rate will lead to even more errors, which can lead to progression or metastasis. The detection of these genomic abnormalities of many types of cancers can play a role in the discovery and development of molecular-based personalized cancer therapies. In this study, we used whole-genomic high-resolution array comparative genomic hybridization (CGH) to identify detailed genomic CNAs on specific loci of chromosomes in SCCs and ACs of NSCLC. Genomic CNA differences between ACs and SCCs were analyzed to establish a CNA signature that may be applied to the diagnosis and clinical management of NSCLC.

2. Materials and methods

2.1. Tumor samples

This study included 47 primary NSCLCs with their corresponding normal tissue samples obtained from the Korea Lung Tissue Bank at Korea University Guro Hospital. Of these 47 primary NSCLC samples studied, 22 were AC and 25 were SCC, which were diagnosed from 2002 to 2010. Two pathologists independently reviewed the hematoxylin and eosin (H&E)–stained tumor slides and validated the histologic diagnosis. We have collected immunohistochemistry (IHC) staining results of p63, thyroid transcription factor-1, surfactant, and cytokeratin 7 for the cases whose staining was performed at the time of diagnosis to confirm the diagnosis (Supplementary Table 1). The stage of each tumor was classified according to the tumor node metastasis (TNM) classification of American Joint Committee on Cancer [10]. All patients had no histories of exposure to either chemotherapy or radiotherapy before surgery. Informed consent was obtained from all participating patients, and institutional review board approval of the ethics committee of the Guro Hospital of Korea University for the study was obtained (No. KUGGR-2011-040). All tumor and paired adjacent normal tissue samples were snap-frozen after surgical resection and stored at −80°C until use. DNA was isolated from the tumor tissue by proteinase K digestion followed by phenol-chloroform extraction according to standard protocols.

2.2. Array CGH

Array CGH was performed by following the manufacturer's protocol of 720k oligonucleotide chip purchased from Roche/NimbleGen System (Madison, WI). Normal control DNA from an individual was used for reference. The DNA from the tumor, the corresponding normal tissue, and the reference samples were labeled with either cyanine-3 or cyanine-5 by random priming (Trilink Biotechnologies, San Diego, CA) and then hybridized to the chip via incubation in the MAUI hybridization system (BioMicro