Lung cancer in which the hypothesis of multi-step progression is confirmed by array-CGH results: A case report

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Abstract. The pathogenesis of lung cancer has not been fully elucidated and biological markers acting as predictors of tumor evolution and aggressiveness remain unidentified. The multi-step hypothesis, suggesting a progression from adenomatous hyperplasia (AAH) to adenocarcinoma (AC) through bronchioalveolar carcinoma (BAC), was highlighted in a previous cytogenetic study performed in a single case. The present study reports the results of an array-comparative genomic hybridization (a-CGH) analysis performed on the DNA obtained from the previously reported case that presented AAH, BAC and AC in one lung. The a-CGH results confirm and support the previous cytogenetic observations with new data, clearly supporting the hypothesis of a multi-step carcinogenic process in the lung.

Introduction

The mechanisms underlying lung cancer pathogenesis and progression remain under investigation. Ground-glass opacity (GGO) lesions, increasingly detected by computed tomography (CT) scanning, can be classified as adenomatous hyperplasia (AAH), bronchioalveolar carcinoma (BAC) or adenocarcinoma (AC). Several studies have suggested that AAH, frequently found in tissue surrounding lung AC, might be a forerunner in the development of AC. Moreover, an increasing genetic complexity associated with lung cancer progression has been demonstrated by means of loss of heterozygosity, fluorescence in situ hybridization, microarrays and immunohistochemistry but the clonal association between AAH, BAC and AC continue to be debated (1-7).

In the present study the array-comparative genomic hybridization (a-CGH) technique was applied on three DNA samples from a patient presenting AAH, BAC and AC in one lung, in order to confirm the previous cytogenetic data for that patient (8), suggesting a clonal evolution of these lesions and the pre-neoplastic nature of AAH.

Case report

A 54-year-old female patient who had never smoked was diagnosed by CT scan at the Humanitas Clinical and Research Center (Milan, Italy) with a lung tumor in the upper right lobe and a GGO in the lower lobe, while the middle lobe appeared to be normal. Written informed consent was obtained from the patient. Histological evaluation lead to a diagnosis of AC with a BAC component in the upper right lobe, BAC in the inferior lobe and AAH in the middle lobe. Following pneumonectomy, a cytogenetic investigation of spontaneous metaphases obtained using a direct method from the three different samples was performed, as previously reported (8,9). The karyotypes showed a clear clonal association among AC, BAC and AAH (8).

An a-CGH study was performed on the DNA isolated from the three lung samples and stored at -20˚C using the Qiagen DNeasy Blood & Tissue kit (Qiagen Inc., Valencia, CA, USA). The samples were then processed using a SurePrint G3 Cancer CGH+SNP 4x180 K microarray kit (Agilent Technologies, Inc., Santa Clara, CA, USA) according to the manufacturer's instructions. Results were analyzed according to Human Genome version 19 (Hg19) (10). The data obtained from the a-CGH study confirmed the hypothesis of multistep progression of the lung cancer that was previously made on the basis of the previously reported, limited cytogenetic results (8). The three different lesions shared losses in 1p, 4p, 9q, 11p, 12p, 14q, 15q, 16p, 16q and 22q (Table I). The common minimal regions of deletions included genes with an established role in lung cancer that regulate the cell cycle or are involved in the maintenance of chromosomal stability and DNA repair (10-14). The most relevant genes included in the deleted regions are listed in Table I. The data obtained support the hypothesis suggested by Bettio *et al* concerning the loss of the cell cycle control

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Table I. Genetic aberrations detected using CGH and SNP microarray analysis in the AAH, BAC and AC samples with the most relevant genes involved in lung tumorigenesis and the percentage of altered cells.

Chromosome imbalances	Size (Mb)	Genes	AAH (%)	BAC (%)	AC (%)
del(1)(p32.3p36.31)	47.5	NBL1-EPHB2-RUNX3-MYCL1-RLF-STIL-CDKN2C-ESP15-ERRFI1-YBX1-BMP8B-COL9A2-PPT1-ZMPSTE24-PPIE-CAP1-HEYL-HPCAL4-TRIT1-OXCT2-NT5C1A-MFSD2-TMCO2-YRDC	15	25	40
del(4)(p14)	2.1	-	30	30	50
del(9)(q31.3q32)	2.0	RGS3-POL3	20	50	70
del(11)(p15.4)	1.9	STK33-AKIP1-WEE1	25	25	30
del(12)(p11.21)	1.8	-	30	25	50
del(14)(q13.1q13.2)	1.1	SNX6	30	30	50
del(15)(q15.1q21.1)	5.7	BUB1B-CASC5-TP53BP1	20	20	40
del(15)(q26.1)	1.9	CRTC3-MFGE8-IQGAP1-FANCI	20	20	35
del(16)(p11.2)	1.7	-	50	50	60
del(16)(q21q22.2)	9.0	CBFB-CDH1	25	25	35
del(22)(q13.1q13.2)	2.7	EP300	20	45	60
dup(1)(q23.1q43)	82.0	PRDX6-MUC1-PRCC-NTRK1-SDHC-LAMC2-CD73-MDM4-PTPN14-TGFB2-AKT3-ARNT-LAMB3	-	60	60
Trisomy 7	159.0	EGFR-DLX5-TWIST1-SEC61G-VSTM2A-TAC1	-	30	70
Monosomy 9	139.3	CDKN2A-CDKN2B-TOPORS-DMRTA1-JAK2-MTAP-XPA-SET-FNBP1-NOTCH1	-	30	40
del(17)(q21.2q21.33)	9.2	ETV4-NR1D1-RARA-TOP2A-BRCA1	-	20	35
Monosomy 22	50.9	NF2-CHEK2-SET5-SMARCB1-EWSR1-ARHGAP8	-	30	40
del(12)(q12q13.3)	12.3	WNT1-ATF1-CDK2-ERBB3	-	-	30
del(12)(q21.33q24.32)	34.1	BTG1-PTPN11	-	-	30
del(16)(p11.2pter)	34.0	CIITA-SOCS1-TNRSF17-ABCC1-PALB2-IL21R-FUS	-	-	30
Monosomy 17	80.7	TP53-NF1-TOP2A-HLF-ERBB2-SOX9-CBX2	-	-	30
Monosomy 19	58.6	STK11-CXCL17-CCNE1-C19orf12-LTBP4-NUMBL-SPTBN4-ADCK4-ITPKC-SHKBP1	-	-	30
del(20)(p12p13)	4.0	RASSF2	-	-	30
del(20)(q12.2q13.33)	23.7	E2F1-SRC-MYBL2-ZNF217-TOP1-CSE1L-BCL2L1-TPX2	-	-	30

CGH, compative genomic hybridization; SNP, single-nucleotide polymorphism; AAH, adenomatous hyperplasia; BAC, bronchoalveolar carcinoma; AC, adenocarcinoma; del, loss of one copy; dup, gain of one copy.

in the AAH sample in which actively replicating cells with a normal karyotype were observed (8).

In addition to the aforementioned abnormalities, the BAC and AC samples showed gain in 1q, loss in 17q, trisomy 7, monosomy 9 and monosomy 22. Notably, these chromosome imbalances involved genes that have previously been demonstrated to play a crucial role in lung cancer (Table I) (10,11,15-20).

Additional alterations were observed only in the AC specimen: Loss in 12q, 20p and 20q, loss of the entire 16p arm and chromosome 17 and 19 monosomy. These regions include genes reported to be frequently deleted in lung neoplasia (Table I) (10,11,21-24).

Discussion

The pathogenesis of lung cancer and the criteria that regulate its progression are not well understood. Several studies have suggested that AAH, frequently found in tissue surrounding lung AC, may be a forerunner in its development but the genetic relationship between these two entities is not yet defined (4,25-27). The present case lends itself well to the investigation of the multistep carcinogenesis hypothesis of the lung because of the presence of three different lesions in the three lobes of the same lung. The a-CGH technique was applied in order to confirm the previous cytogenetic observations that were limited by the

low proliferation rate of the tumor cells, and the poor resolution and incompleteness of metaphase spreads, which allowed the identification of only a few clonal aberrations. Moreover the a-CGH analysis allowed a more detailed characterization of the genomic imbalances present in the three lesions. The data appear to support the pre-neoplastic nature of the AAH lesion that presented several genetic alterations shared with the BAC and AC samples. The chromosomal regions showing recurring imbalances encompass genes with already demonstrated critical roles in lung cancer development and progression: Tumor suppressor genes, genes controlling the cell cycle, differentiation and apoptosis (10-24). The presence of chromosome abnormalities shared by all the samples and the increase in the number of rearrangements and percentage of altered cells from AAH through BAC to AC clearly correlate with a clonal evolution confirming the multi-step process in lung cancer.

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