Nonrandom DNA copy number changes related to lymph node metastases in squamous cell carcinoma of the lung

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Received April 2, 2008

Lung cancer is one of the most common malignancies and cancer-related death worldwide. Lymph node metastasis is the main cause of treatment failure. Although many studies were performed to evaluate genetic events associated with development and progression of lung cancer, molecular mechanism still remains poorly defined.

In the present study, using comparative genomic hybridization (CGH) technique, we described the pattern of DNA copy number changes in a cohort of 42 primary squamous cell carcinomas (SCC) of the lung. A direct comparison of non-metastatic ($T_xN_0M_0$) and metastatic ($T_xN_{1-2}M_0$) tumors was performed to define chromosomal imbalances related to lymph node metastases.

Some genetic alterations were observed more frequently in metastatic than in non-metastatic tumors, including losses at 11q, 16p, 16q, 19p and gains at 4q, 7q, 12p, 13q, 18p. The gain at 7q with the smallest common altered region 7q31.2-q32, was found to be directly associated with lymph node involvement (p=0.0407). We suggest that the established chromosomal region harbors two putative tumor suppressor genes *WNT2* and *c-Met*. An overexpresion of these genes seems to be involved in inducing the invasive growth and metastatic potential of SCC of the lung.

Key words: Squamous cell lung cancer, lymph node metastases, comparative genomic hybridization, gain at 7q31.2-q32, WNT2, c-Met

Lung cancer is one of the most common malignancies and cancer-related deaths worldwide [1]. Epidemiological data from Poland indicate that each year about 20.500 individuals develop lung cancer, that makes morbidity rate one of the highest in the world (for males 77.9/100 000 individuals and for females 13.2/100 000). Consequently, lung cancer is the most common cause of cancer death in males (34.9%), and the second most frequent cancer in females (10.1%) that implicates the first place among all tumor related deaths in Poland [2].

Non-small cell lung cancer (NSCLC) is the most frequent histological type of lung cancer accounting for 75-80% of cases. Approximately 30-40% of all NSCLC are classified as squamous cell carcinoma (SCC). According to the follow-up data, 5-year survival rate of NSCLC patients is one of the lowest of all human cancers [3–5]. The low effectiveness of treatment is connected with the late detection of lung cancer, usually in advanced clinical stage. However, the main cause of treatment failures is associated with lymph node recurrences [6]. Percentage of 5-year survival rates for patients with diagnosed lymph node metastases is estimated for 10-60%, while the survival rate in cases without metastases reaches 75% [7–10].

Many studies were performed to evaluate genetic events associated with development and progression of SSC of the lung. Frequent chromosome changes including losses of 1p, 2q, 3p, 4p, 4q, 5q, 6q, 8p, 9p, 10q, 11p, 13q, 17p, 18p, 18q and 21q, as well as over-representations of 1q, 3q, 5p, 8q, 11q, 12p, 17q, 19p, 20q and 22q were reported [11–15]. Several tumor suppressor genes (TSGs) and proto-oncogenes have been mapped, in chromosomal regions commonly deleted and gained in lung cancer, respectively, and it has been shown, that these genes contribute to lung cancer development and progression [16, 17]. However, molecular mechanism of lung cancer progression and lymph node metastasis formation is still not sufficiently defined.

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The aim of our study was to describe a pattern of DNA copy number changes related to lymph node metastasis in squamous cell carcinoma of the lung using comparative genomic hybridization (CGH) technique. The established aberrant chromosome regions were further analyzed for genes which may be involved in metastases formation.

Materials and methods

Tissue specimens. Lung tumor tissues were collected from 42 patients who underwent surgery for lung SCC. Assembled cohort consisted of 11 primary non-metastatic tumors ($T_xN_0M_0$) and 31 metastatic tumors ($T_xN_{1-2}M_0$). Clinical and histological data are summarized in Table 1.

Tissues samples were obtained from the Department of Pathomorphology, Silesian Medical Academy, Zabrze, Poland (32 tumor samples of archival material stored as paraffin blocks) and the Department of Thoracic Surgery, University of Medical Sciences, Poznan, Poland (10 fresh tumor samples). Tumor tissues specimens were divided into two parts: one for histopathological examination and other for DNA extraction. Tumors were classified according to the WHO histological typing of lung tumors [18] and staged according to the TNM classification of malignant tumors [19]. All tumors selected to the studies were classified as squamous cell carcinoma.

Table 1. Clinicopathological characteristic of studied samples

Age	
Range	40-74
Mean age (±SD)	59.7 (±9.41)
Sex	
Male	38 (90.4%)
Female	4 (9.6%)
Histologic type	
Squamous cell carcinoma	42 (100%)
Stage	
I	11 (26.2%)
II	13 (30.9%)
III	16 (38.1%)
S_x	2 (4.8%)
T values	
T_1	8 (19.0%)
T_2	28 (66.6%)
T_3	5 (11.9%)
T_4	0
T_x	1 (2.4%)
Lymph node involvement	
N_0	11 (26.2%)
N_1	15 (35.7%)
N_2	12 (28.5%)
N_x	4 (9.6%)
Histological differentiation	
G_1	6 (14.3%)
G_2	27 (64.3%)
G_3	8 (19.0%)
G_x	1 (2.4%)
Distant metastases	
M_0	42 (100%)
M_1	0

Sx, Tx, Nx, Gx- data not available

DNA isolation. DNA from tumor specimens was isolated after malignancy had been histologically confirmed and contamination by normal cells (≥ 30%) had been eliminated by an experienced pathologist. Paraffin-embedded samples were deparaffinized by xylene washing, then hydrated. DNA was isolated by proteinase K (Sigma, USA) digestion followed by standard protocols with phenol-chloroform extraction and ethanol precipitation.

Comparative genomic hybridization and image analysis. Previously published CGH protocol was used [20] with some modifications. Briefly, tumor and normal reference DNA were labeled by nick translation with biotin-16-dUTP (Roche Diagnostic GmbH, Mannheim, Germany) and Tetramethylrhodamine-5dUTP (Roche Diagnostic GmbH, Mannheim, Germany), respectively and hybridized to normal metaphase chromosomes. Biotin signals were detected using avidin-fluorescein isothiocyanate (FITC) (Vector Laboratories, Inc, Burlingame, CA). From each metaphase spread three-color images (red for reference DNA, green for tumor DNA and blue for metaphase chromosomes counterstained with 4',6-diamidino-2-phenylindole, DAPI), were collected using CCD camera mounted on an Axiopoth fluorescence microscope (Opton, Germany), and analyzed using ISIS digital analysis system (MetaSystem Hard & Software, Altlussheim, Germany). For each tumor specimens 8 to 10 metaphases were analyzed. The constitutive heterochromatic regions at 1q, 9q and 16q, Y chromosome, as well as acrocentric regions of 13p, 14p, 15p, 21p were excluded from the CGH analysis.

Statistical analysis. The mean copy-number imbalances, as well as gains and losses were analyzed using the Student's *t*-test. Correlations between chromosomal aberrations in metastatic versus non-metastatic tumors were calculated by χ^2 test or Fischer's exact test. The differences were considered significant when the value of probability (P) did not exceed 0.05.

Results

DNA copy number imbalances in 42 primary tumors detected by CGH. CGH technique was applied to whole genome analysis of chromosomal aberrations in the collected material. The graphical representation of genetic changes detected in all of 42 primary tumor specimens is displayed in Figure 1.

The clinical data and chromosome imbalances established in the whole group of primary tumor samples (42 specimens) by CGH analysis are shown in Table 2. Chromosome abnormalities were detected in all studied primary tumor samples. The total number of DNA copy changes was 435 ranging from 1-24 with the mean number of chromosome aberrations equal to 10.3±5.89 per sample. Gains were more frequent than losses: 241 (5.7 per case) and 194 (4.6 per case), respectively, however the difference was not statistically significant (p=0.084). In the group of primary tumors the most prevalent losses with an incidence over 30% were found at: 1p (33/42;

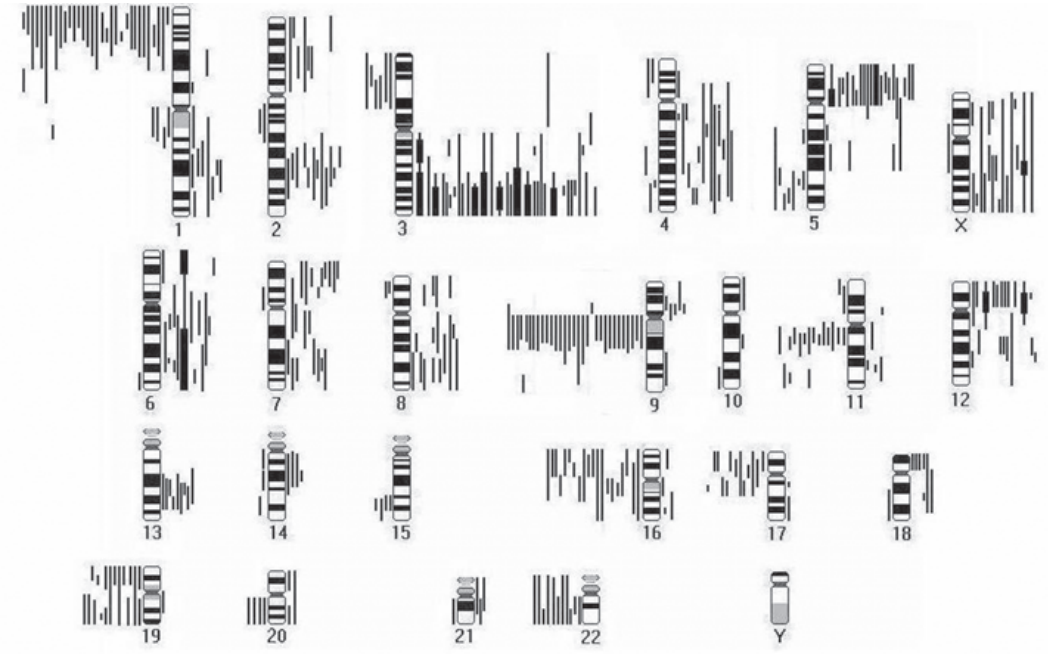

Figure 1. Summary of comparative genomic hybridization abnormalities identified in 42 primary lung squamous cell carcinomas. Each vertical line represents a single genetic aberration observed in a single tumor specimen. Losses are shown on left and gains on the right of individual chromosomes. High-level amplifications are shown as thick lines.

76%), 9q (31/42; 74%), 11q (17/42; 40%), 16p (15/42; 36%), 16q (14/42; 33%), 22q (13/42; 31%). Gains were found at: 3q (33/42; 76%), 4q (17/42; 40%), 5p (19/42, 45%), 6q (13/42; 31%), 7q (13/42; 31%), Xq (14/42; 33%). DNA amplifications were observed in thirteen regions; the most frequent amplifications were found at 3q (9/42; 21%) including the following subregions: 3q13.1-q21 (one case), 3q23-qter (one case), 3q24-qter (one case), 3q25-qter (one case), 3q25-qter (one case), 3q26.1-qter (four cases), 3q26.1-q28 (one case) and in 5p (one case), 5p11.2-p13 (one case), 6p22-pter (one case), 6q16.3-qter (one case), 12cen-p12.2 (two cases), Xq21.3-q22.2 (one case).

Comparison of DNA copy number imbalances in non-metastatic and metastatic tumors. To define chromosomal regions potentially associated with the metastatic phenotype, a comparison of non-metastatic versus metastatic tumors was performed.

A global pattern of chromosome imbalances in non-metastatic primary tumors ($T_xN_0M_0$) compared to metastatic tumors ($T_xN_{1-2}M_0$) is shown in Figure 2A and 2B, respectively. The mean number of chromosomal imbalances was higher in tumors with lymph node metastases than in non-metastatic tumors, however the difference was not statistically significant (10.94±5.29 versus 8.72±5.62, p=0.2492 respectively). Gains and losses occurred with a similar frequency in both groups (6.03±4.31 and 4.90±2.18 versus 4.91±3.70 and 3.81±2.27, respectively).

For most chromosome arms aberration profile was common or very similar in both studied groups of tumors, however some alterations were observed more frequently in metastatic than in non-metastatic tumors (Figure 3). The following alterations occurring considerably more frequently in metastatic tumors seem to be associated with the process of metastasis to the adjacent lymph nodes: losses at 11q (45.2% in $T_xN_{1-2}M_0$ compared to 18.2% in $T_xN_0M_0$, respectively), 16p (38.9% / 27%), 16q (41.9% / 9%), 19p (25.8% / 18%) and gains at 4q (45.2% / 27%), 7q (35.5% / 0), 12p (29% / 18.2%), 13q (29% / 9%), 18p (16% / 0). However, the only significant difference (p=0.0407) between metastatic and non-metastatic tumors was found for a gain at 7q.

To narrow down the regions of interest, the smallest common regions of losses and gains were assessed as follow: losses at 11q13 (12/14; 85.7% of all observed losses at 11q), 16p11.2-p12 (11/12; 91.7%), 16q13 (11/11; 100%) and gains at 7q31.2-q32 (7/11; 63.6%), 13q 21.3-q22 (7/8; 87.5%). Using the UCSC Genome Browser on Human March 2006 Assembly (http://genome.ucsc.edu), several putative TSGs and proto-oncogenes were proposed in the established chromosomal regions: for losses at 11q13 – *MEN1* (11q13.1), *BAD* (11q13.1), *BRMS1* (11q13.2), 19p – *LKB1* (19p13.3), and for gains at 4q – *RCHY1* (4q13.1-13.3), *bFGF* (4q26-27), 7q31.2-q32 – *WNT2* (7q31.2), *c-Met* (7q31.2), *POT1* (7q31.3), 12p – *KRAS* (12p12.1), *SSPN* (12p12.1), *CCND2* (12p13.3), *FGF23* (12p13.3), *FGF6* (12p13.3), 13q 21.3-q22 – *KLF5* (13q22.1). Taking into account the above mentioned statistical analysis the oncogenes *WNT2*, *c-Met*, and *POT1* anchored at 7q31.2-3 seem to be the primary candidates involved in metastasis formation in lung squamous cell carcinoma.

Table 2. DNA copy number changes detected by comparative genomic hybridization in 42 primary lung squamos cell carcinomas.

Primary tumor	Sex	Age	TNM	G	Total number of imbalances	Losses	Gains [a]
SQ 17	M	64	$T_1N_0M_0$	3	12	1p34.1-pter; 9cen-q21.3; 16p; 17p; 19p13.1-p13.2; 19q13.1-ter; 22	2q24.3-q33; 4q24-q26; 5q21-q23.2; 6cen-q16.3; X
SQ 18	M	50	$T_2N_0M_0$	2	15	1p; 3p14.1-pter; 5q14-qter; 9cen-q21.2; 19p13.2-pter	1q32.1-q41; 2p21-pter; 2q32.1-q32.2; 3q24-qter/**26.1-qter**; 5p; 6q22.3-qter; 7p14-pter; 8q; 9p12-p13; 12pter-q13.1/**12p12.3-cen**
SQ 19	M	57	$T_2N_0M_0$	3	11	1p31.2-pter; 2q13-q21.1; 9q33-qter; 21q; 22q13-qter	5p14-q12; 7p21-pter; 12p12.3; 16q13-qter; Xp21.1-p22.1; Xq13-q21.1
SQ 21	M	68	$T_1N_0M_0$	2	19	1p33-pter; 15q24-qter; 16p; 17p; 17q21.1-q21.2; 19q; 20q. 22	1q31; 2q24.3-q31; 3q26.1-q26.2; 4p14.2-q32; 5cen-q23.2; 6p12-q23.1; 8q13-q21.1; 12q15-q21.3; 13q14.3-q31; 14q21. X/**Xq21.3-22.2**
SQ 23	M	48	$T_2N_0M_0$	3	2	9cen-q21.2;	3q25.3-qter
SQ 25	M	62	$T_2N_0M_0$	3	4	1p31.1-pter; 19q	3q25.1-qter; 5p;
SQ 26	M	73	$T_1N_0M_0$	2	9	1p34.1-pter; 1q21.3-q22; 15q23-q26.1; 22	3p12-q13; 3q25.3-q26.1; 4q22-q33; 6q14-q16.2; X
SQ 30	M	71	$T_2N_0M_0$	2	11	4q25-q28; 9cen-q21.3; 11q22.3-qter	1q31-q32.1; 3q; 5p13.2-p14; 6p22.3-p24; 8p21.2-pter; 8q21.1-qter; 18cen-q22; 20
SQ 31	M	nd	$T_1N_0M_0$	2	8	1p21-p22.3; 9cen-q21.3; 11q12-q14.1	3p; 3q13.2-q13.3; 3q24-qter; 5p; 12q21.3-q22
LG29-1A	M	45	$T_1N_0M_0$	2	1	9cen-q21.3	no changes
LG10-1A	M	63	$T_2N_0M_0$	3	4	1p34.3-p36.1; 9p13-q21.3; 16p-q21	3q26.1-qter
SQ 1A	M	68	$T_2N_1M_0$	2	13	1p36.1-pter; 3p14.1-pter; 4q31.2-q31.3; 5q31.3-q32; 9cen-q21.3; 11q12-q13.4;	3q/**3q13.1-q21/3q24-qter**; 4q13.3; 5p/**5p13.3-cen**; 6p21.3-pter; 8q22.2-qter; 18p; 21pter-q22.1
SQ 4A	M	74	$T_2N_1M_0$	2	15	1p31.1-pter; 2q12-q22; 4p15.1-pter; 9cen-q21.1; 16; 22q	3q21-qter; 4q11.2-q28; 5cen-p14; 6q15-q24; 7q22-q33; 9p13-p21; 12pter-q13.1; Xp11.3-p22.1; Xq22.2-qter
SQ 5A	M	63	$T_2N_1M_0$	2	3	1p31.3-pter; 9cen-q21.3	4q11.2-q13.3
SQ 32A	M	56	$T_2N_1M_0$	2	12	1p32.2-p35; 9cen-q22.1; 11q12-q13.3; 16p12-q23; 17pter-q21.3; 19p; 19q13.1-qter; 22q11.1-q13.1	4q28-q31.3; 4q32-q34; 13q21.1-q32; 18p
SQ 33A	M	66	$T_{nd}N_1M_0$	nd	9	1p31.1-pter; 11cen-q14.1	2p; 3q24-qter/**3q26.1-qter**; 7p15.1-q21; 8q21.1-q21.3; 12p; 12q21.3-q22; 13q21.2-q32
SQ 34A	M	63	$T_3N_1M_0$	2	4	1p31.3-pter; 5q31.3; 22q13.1-qter	3q24-qter
SQ 35A	M	51	$T_2N_1M_0$	1	9	1p31.3-pter; 1cen-q21.3; 11q12-q13.5; 16p; 19p	3q13.1-q13.3; 3q25.2-qter; 5p13.2-p14; 18p;
SQ 36A	M	69	$T_1N_1M_0$	1	9	1q21-q23; 11cen-q13.4; 17p; 19; 20q; 22q13.1-qter	5q13.3-q14; 8q21.3-q24.1; Xq26-q27
SQ 37A	M	74	$T_2N_1M_0$	2	7	1p32.1-pter; 9cen-q21.3; 19p13.1-pter; 22	3q26.2-q27; 4q25-q27; 13q21.3-q22
SQ 38A	M	73	$T_1N_1M_0$	1	18	1p32.1-pter; 9cen-q21.3; 11q13.2-q13.5; 15q23-q25; 16p13.1-q13; 17p12-q21.1; 19q; 20q; 22	1p21; 2q32.1-q35; 3q26.1-q26.2; 4p14-q31.2; 5q21-q23.3; 6q14-q16.1; 6q23.1-q23.3; 12q14-q21.3; 13q22-q32
LG1-1A	M	58	$T_2N_1M_0$	2	22	1p35-p36.2; 3p14.2-pter; 4p; 4q31.3-qter; 5q14-q15; 5q31-qter; 6q25.1-qter; 9p12-p13; 10q24.3-qter; 11p12-p15.3; 18q12.3-qter	1cen-q23; 1q25-q32.2; 3q; 7cen-q21.1; 10q21.3-q22.2; 11cen-q14.1; 11q22.3-q23.1; 12p-q13.3/**12p12.3-cen**; 14q11.2-q24.1; 16cen-q13; Xp21.3-qter
LG2-1A	M	58	$T_2N_1M_0$	3	5	1p33-p34.2; 9cen-q21.1; 16q11.2-q13	9p23-pter; 12p
LG14-1A	M	50	$T_3N_1M_0$	2	7	1cen-q22; 1p32.3-pter; 9cen-q21.3; 16cen-q21; 19p13.3-qter; 22	4q32-q34
SQ 3A	M	64	$T_2N_1M_0$	2	13	5q33.2-q34; 8p21.3-p23.1; 9cen-q22.1; 16cen-q12.1	1q24-q31; 2p23-pter; 2q24.3-q32.3; 3q25.2-qter; 7p13-pter; 7q11.2-q22; 7q31.3-qter; 11q22.1-q22.3; Xq22.2-qter
LG 22-1B	M	64	$T_2N_1M_0$	2	10	1p31.3-pter; 1cen-q21.3; 9cen-q21.1; 16q11-q12.2; 17cen-p11.2	3q24-qter; 4p12-p15.3; 4q21.3-q22; 5cen-p15.2; 7q31.3-q35;
LG15-1B	M	58	$T_3N_1M_0$	2	21	1p32.3-p34.3; 5q33.3-qter; 11q12-q13.4; Xp11.3-q22.1	1q23-q31; 1q41-qter; 2p13-p21; 2q24.3-q33; 3q25.2-qter/**3q26.1-qter;** 7p; 7q11.2-q21.2; 7q32-q35; 8p12-p22; 8q22.3-qter; 9cen-p13; 9q22.1-q34.1; 12p11.2-p13.3; 14q11.1-q22; 16p12-pter; 16q23; 20p11.2-pter
LG13-1A	M	44	$T_2N_1M_0$	2	6	1p32.3-p36.1; 9cen-q21.1; 11q12-q13.3; 16cen-q13	7p15.1-p21; 13q31-q33;
SQ 6A	M	49	$T_2N_2M_0$	2	10	1p31.1-pter; 3p14.3-p22; 9cen-q21.2; 11q12-q14.3	3q/**3q24-qter**; 5cen-p15.1; 7p13-p14; 7q31.1-q35; 18p; 18q21.1-q21.2

Table 2. Continued

Primary tumor	Sex	Age	TNM	G	Total number of imbalances	Losses	Gains [a]
SQ 7A	M	46	$T_2N_2M_0$	2	14	1p31.3-pter; 9cen-q22.3; 11q13.1-q13.4; 16; 17q12-q21.3	2q23-q31; 2q32.1-q33; 5cen-p13.3; 6p21.1-q16.1; 6q23.2-q24; 7p15.1-p21; 7q31.3-q32; 8q13-q21.1; 12q15-q21.3
SQ 9A	M	66	$T_2N_2M_0$	1	10	1p32.2-pter; 3p14.1-p21.3; 9cen-q34.1; 11q13.1-q21; 11q23.2-qter; 16; 19p13.1-pter	2p; 3q; 5p
SQ 12A	M	58	$T_2N_2M_0$	2	24	3p21.3-p24.2; 7cen-p14; 8p21.1-p23.1; 14cen-q13; 14q21-q23; 16cen-q21	1q12-qter; 2q22-q37.1; 3q/**3q23-qter**; **5p**; 6p/**6p22-pter/6q16.3-qter**; 8cen-q21.3; 8q22.3-q24.1; 9p13-pter; 10p; 12q; 17q12-q21.1; 17q22-qter; 19cen-q13.2; 20q12-qter; 21q; Xp21.1-p22.1; Xq21.2-q23; Xq27-qter
SQ 13A	M	66	$T_2N_2M_0$	2	13	1p33-pter; 9cen-q21.2; 16p; 17pter-q21.3; 19; 20q; 22	1p21.3-p22.3; 4p14-q33; 5q15-q23.3; 8p21.3-pter; 12q15-q21.3; 13q21.1-q32
SQ 39A	M	44	$T_2N_2M_0$	2	12	1p35-pter; 9cen-q21.3; 17cen-p12; 19q13.2-qter	2p14-p21; 2q24.1-q35; 3q25.1-qter/**3q26.1-q28**; 5p; 6q14-q25.3; 8q22.2-q24.1; 12p; 14q11.2-q22
SQ 40A	M	66	$T_2N_2M_0$	2	15	1p32.3-pter; 9cen-q22.3; 11p15.1-pter; 16p11.2-p13.2; 17p; 17q12-q21.3;	2p13-p21; 2q31-q32.1; 4q; 5pter-q12; 8p12-qter; 12p; 12q15-q22; 13q21.3-q33; Xq13-q23
SQ41A	M	54	$T_2N_2M_0$	3	11	1p35-pter; 9cen-q21.3; 11q12.1-q13.3; 16pter-q21	3q24-qter; 5p; 7p21-pter; 13q21.3-q22; 18pter-q22; Xp11.2-p22.1; Xq21.1-qter
SQ10A	M	40	$T_1N_2M_0$	3	4	9cen-q21.2; 16p11.2-p12	4q12-q21.1; Xp22.2-qter
LG19-1B	M	50	$T_2N_2M_0$	2	9	1p32.3-p36.2; 19q13.3-q13.4	3q24-qter/**3q26.1-qter**; 5cen-p15.1; 6q12-q15; 7p15.1-pter; 7q31.1-q36; 11q21-q23.3; 12p
SQ 42A	M	69	$T_2N_2M_0$	1	12	1p32.1-pter; 9cen-q21.1; 16p13.1-pter; 17q11.2-q21.3; 19p	3q25.1-qter; 5p13.3-p15.2; 6q23.1-q24; 8q21.1-q23; 11p12-p13; 12p; 13q22-q32
SQ 43A	M	68	$T_3N_2M_0$	2	11	5q23.3-q31.3; 9cen-q21.1; 11q13.2-q14.1	2q32.1-q36; 3q13.2-q13.3; 3q25.1-qter; 4q13.1-q24; 4q31.1-q33; 5p11.2-p14; 21pter-q21; Xq23-qter
SQ 44A	M	53	$T_2N_2M_0$	1	8	1p31.1-pter; 9cen-q21.1; 11q23.3-q24; 14q24.3-q32.2; 15q23-q25; 16p; 22q	3q;
SQ 30-1A	M	64	$T_3N_2M_0$	2	3	9cen-q21.3; 16cen-q13	3q25.3-qter

Abbreviaions: TNM – tumor-nodes-metastases classification of the Internatonal Union Against Cancer, G- grading, M-male, F-female, nd-no data.

[a] High-level amplifications (≥1.5) are in bold.

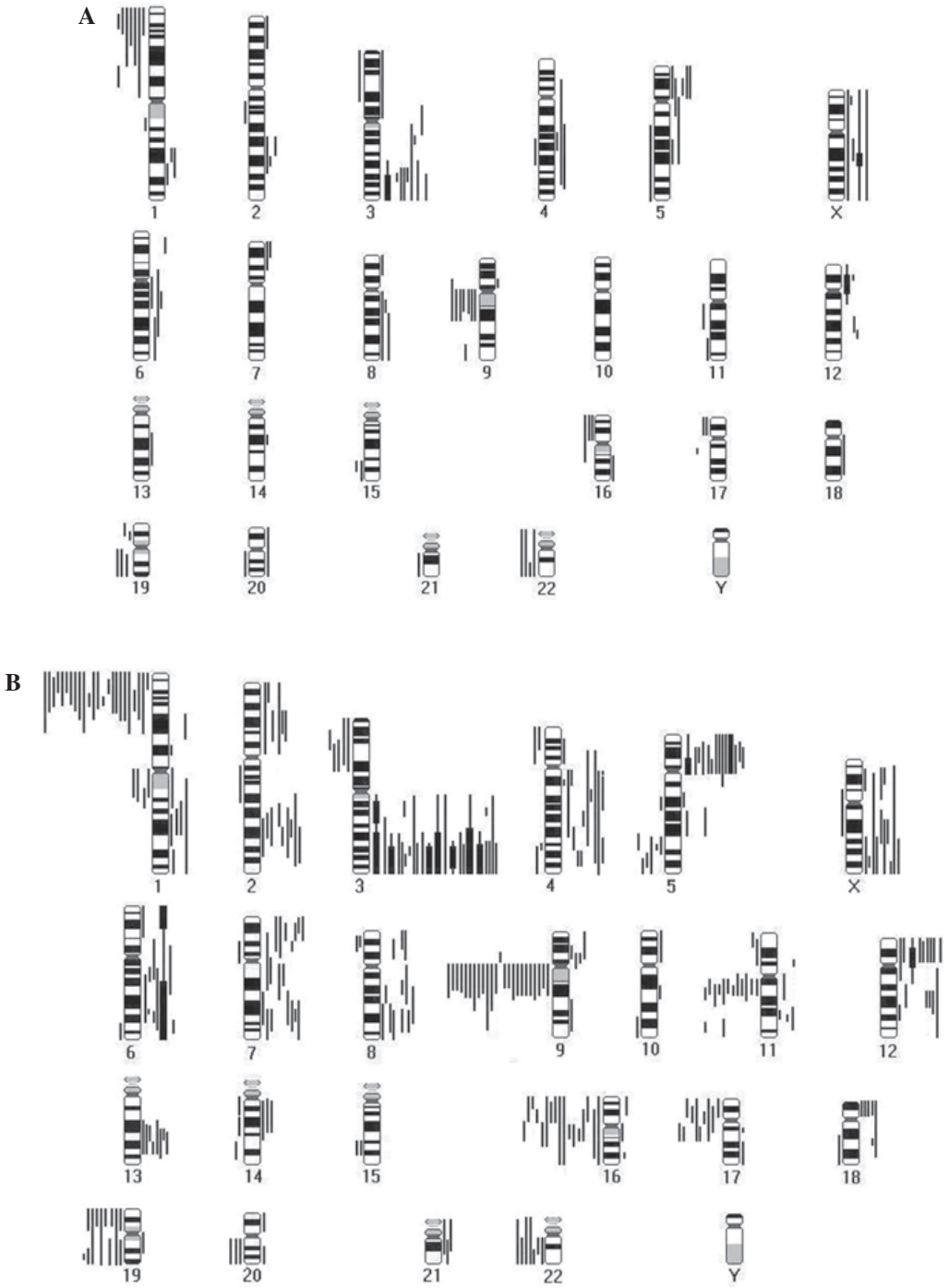

Fig. 2. Summary of comparative genomic hybridization abnormalities identified in primary lung squamous cell carcinomas. Genetic changes detected in non-metastatic ($T_xN_0M_0$) (Fig. 2A) and metastatic tumors ($T_xN_{1-2}M_0$) (Fig. 2B). Each vertical line represents a single genetic aberration observed in a single tumor specimen. Losses are shown on left and gains on the right of individual chromosomes. High-level amplifications are shown as thick lines.

Discussion

Lymph node metastasis is one of the most critical reason of treatment failures in NSCLC [21]. Although many studies were performed to understand the metastatic process, molecular mechanism still remains poorly defined. In the present study a direct comparison of chromosomal aberrations between non-metastatic ($T_xN_0M_0$) and metastatic primary tumors

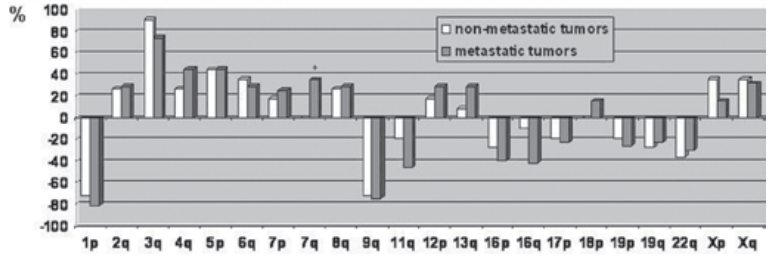

Figure 3. Frequency of DNA copy number changes in non-metastatic and metastatic tumors in selected chromosome arms (gains – upper panel and losses – bottom panel). NOTE: + Difference statistically significant, p=0.0407

($T_xN_{1-2}M_0$) was conducted to highlight differences that may be associated with an appearance of lymph node metastases.

Some specific alterations were detected more frequently in tumors with lymph node metastases than in non-metastatic tumors. Among them, the gain at 7q was observed only in $T_xN_{1-2}M_0$ tumors (p=0.0407), what may suggests that this chromosomal aberration is a late cytogenetic event associated with tumor progression and metastasing. The smallest common amplified region encompassing 7q31-q32 (7/11; 63.6%) was defined.

Several genes were mapped in this region, including *WNT2* (7q31.2) and *c-Met* (7q31.2) which may be considered as putative activated proto-oncogenes. *WNT2* gene is a member of the *WNT* gene family consisted of structurally related genes encoding the secretion signaling proteins implicated in apoptosis inhibition. Wnt-2 has been implicated in human carcinogenesis by up-regulation and altering an activation of Wnt/β-catenin signaling pathway [22, 23]. It has been already shown that blocking of Wnt signaling can cause antitumor effects by inducing apoptosis in human cancer lines [22, 24]. The proto-oncogene *MET (c-MET*) product is the hepatocyte growth factor receptor and encodes tyrosine-kinase activity The *c-Met* overexpression has been shown in NSCLC and was significantly associated with angiogenesis, tumor growth and invasion [25, 26]. Cheng *et al.* [27] have found, that overexpression of *c-Met* in tumor tissue, as well as high level of *c-Met* mRNA circulating in peripheral blood of NSCLC patients, are correlated with the T value, lymph node involvement and early recurrence. Recently, De Herdt *et al.* [28] have shown that an overexpression of *c-Met* correlates with an invasive growth in a case of head and neck SCC. Our findings and the discussed results provide a strong evidence, that both *WNT2* and *c-Met*, may be considered as genes putatively associated with tumor invasion and dissemination in lung SCC.

Interestingly, we found deletion of 11q and gain at 13q as frequent events in metastatic tumors (45% and 29%, respectively), that is in contrast with the formerly reported general profile of chromosomal aberration for NSCLC. Gain at 11q13 is commonly described as associated with amplification of *CCND1* gene in SCC of the lung, while 13q, including 13q14, is lost and leads to an inactivation of *RB1* gene [13, 29]. Our previous studies in the field of chromosomal aberrations in laryngeal squamous cell carcinoma, confirmed that amplification at 11q13 [30], as well as loss at 13q [31] are frequently observed cytogenetic events committed with larynx cancer progression.

Currently presented results may suggest, that these regions contain also some other target gene(s) (putative TSG's and proto-oncogenes, respectively) associated with tumor progression and nodal invasion. A potentially relevant genes are: (i) *MEN1* (11q13.1), a putative tumor suppressor gene encoding menin, associated with a syndrome known as multiple endocrine neoplasia type 1. Recent data suggest that interactions between menin and menin-interacting proteins play a role in physiological regulation of cell growth, control of the cell cycle and genome stability [32] (loss of heterozygosity (LOH) using three mikrosatellite markers adjacent to *MEN1* gene were analyzed, however low level of informative rate disabled further investigations – data not shown), (ii) *BRMS1* (11q13.2), it was shown that this gene reduces the tumor metastatic potential of human breast cancer [33] and melanoma [34], (iii) *KLF5* gene (13q22.1) encodes a member of the Kruppel-like factor subfamily of zinc finger proteins, recognized as transcription factor and cell growth mediator. Tong *et. al* [35] have found a direct correlation between overexpression of *KLF5* and cell proliferation *in vivo*, as well as shorter disease-free survival and overall survival in breast cancer patients.

Molecular mechanism of cancer progression and lymph node metastases formation in SCC of the lung remains still not sufficiently defined. In this study, we identified several nonrandom chromosomal imbalances and proposed new regions and candidate genes (TSGs and proto-oncogenes) which may be involved in cancer progression and lymph node metastases. We indicate that gain at 7q31-q32 and *WNT2* and/or *c-Met* genes overexpresion may be an important cytogentetic and molecular events associated with lymph node involvement in SCC of the lung.

This work was supported by the Polish State Committee for Scientific Research with the grant no. 2 P05A 011 26. The technical assistance of Mariola Zawada and Magdalena Kostrzewska–Poczekaj is gratefully acknowledged.

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