Increased Insulin-Like Growth Factor 1 Receptor Protein Expression and Gene Copy Number in Small Cell Lung Cancer

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Purpose: Identification of new therapies in small cell lung cancer (SCLC) is urgently needed. Insulin-like growth factor 1 receptor (IGF1R) is a tyrosine kinase receptor implicated in the pathogenesis of several malignancies and is potentially an attractive target for anticancer treatment. Knowledge about IGF1R protein expression, gene copy number, and the prognostic relevance of these features in SCLC is limited.

Methods: We analyzed IGF1R protein expression and gene copy number in primary tumors from 90 patients with SCLC (67 men and 23 women) who underwent pulmonary resection. IGF1R expression assessed by immunohistochemistry with H scores from 0 to 400 was evaluable in 84 patients and *IGF1R* gene copy number assessed by silver in situ hybridization technique in 81 patients.

Results: Median H score for IGF1R protein expression was 88 (range, 0–400), and the proportion of positive immunostaining using cutoff H score of 10 was 74%. Increased *IGF1R* gene copy number (an average of four or more copies per cell) was found in 15 cases (18.5%), five of whom (6.2%) showed gene amplification. There was a significant correlation between protein expression and gene copy number ($r = 0.49$, $p < 0.005$). IGF1R expression and gene copy number did not associate with clinicopathological factors such as patient age, tumor size, lymph node involvement, stage, and survival.

Conclusions: SCLC is characterized by frequent high-IGF1R protein expression, increased gene copy number, and occasional occurrence of true gene amplification. These features may have important implications for future anti-IGF1R therapeutic approaches.

Key Words: Lung cancer, IGF1R, Protein expression, Gene copy number, Prognosis.

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SCLC comprises approximately 15% of new lung cancer diagnoses in the United States[1] and approximately 10 to 20% in Europe. Although the incidence of small cell lung cancer (SCLC) is slowly decreasing, the cure rates have increased only slightly over the last decades and remain elusive.[1] Median survival of patients presenting with limited disease (LD) and extensive disease (ED) is approximately 16 to 24 months and 6 to 12 months, respectively. Novel treatment approaches considering biologic characteristics of this tumor are, therefore, urgently needed.

The insulin-like growth factor (IGF) pathway regulates several important cell functions, including cell growth, proliferation, survival, and invasion. The extracellular pathway components include two ligands (IGF1 and IGF2, produced under the control of growth hormone), their binding proteins (IGFBP1-6, governing ligand bioavailability in the circulation, tissue, and cell), and two cell membrane receptors (IGF1R and IGF2R).[2] IGF1R is structurally related to insulin receptor and has tyrosine kinase activity capable of downstream signaling through RAS/RAF/mitogen-activated protein kinase pathway and phosphatidylinositol-3-kinase-Akt pathway, whereas IGF2R acts as a decoy receptor. Increased IGF1R activity results in up-regulation of survivin expression,[3] which could be a potential mechanism of chemoresistance in SCLC.

Because of the paucity of existing data on IGF1R abnormalities in SCLC, the aim of this study was to evaluate IGF1R protein expression and gene copy number in this tumor type in relationship to clinical characteristics and survival. Because adequate primary tumor specimens of

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SCLC are difficult to obtain and typically small, we used previously described primary tumor specimens collected during a 20-year period from series of patients with LD who underwent pulmonary resection.[4]

PATIENTS AND METHODS

Patient Population

Archival formalin-fixed paraffin-embedded tumor samples were obtained from 90 patients with SCLC who underwent pulmonary resection between 1982 and 2002 at Medical University of Gdansk. Because of difficulties in obtaining reliable biopsy material for histopathological examination, the diagnosis of SCLC in this group was established only after the pulmonary resection. In all patients, surgery was followed by one of the standard chemotherapy regimens. Patient clinical characteristics are shown in Table 1. Median follow-up was 86 months (range, 1–211 months), with a 2-year survival probability of 43% and a median survival of 17.8 months.

Tissue Microarray Preparation

On each paraffin-fixed block, a morphologically representative SCLC tumor area was identified under the microscope by a pathologist, using a stained hematoxylin and eosin section on a glass slide as a guide. Tissue microarray was constructed using manual MTA I Beecher Instrument. Three 0.6 mm cores from different tumor areas were taken for each patient.

IGF1R Protein Expression

Immunohistochemistry evaluation, on 4 μM sections, was done using the Ventana G11 (CONFIRM, Ventana Medical Systems, Tucson, AZ) anti-IGF1R antibody following the manufacturer's instructions. Briefly, the staining was performed on the Ventana BenchMark XT autostainer using the ultraView detection kit, and the primary antibody was incubated for 16 minutes. The score was determined according to the "hybrid scoring system" (H score) criteria, which sums the products of the five staining intensity categories (0–4) by the percentage of positive cells (0–100%). Thus, the final immunohistochemistry score ranged from 0 to 400. Two certified pathologists and one reader independently scored each core.

TABLE 1. Patient Characteristics

Characteristics	
Age (yr)	
Median (range)	57 (37–82)
≥60, *n* (%)	38 (42)
Gender, *n* (%)	
Female	23 (26)
Male	67 (74)
Pathological stage, *n* (%)	
IA	6 (7)
IB	26 (29)
IIA	5 (6)
IIB	13 (14)
IIIA	24 (27)
IIIB	12 (13)
Unknown	1 (1)
Surgery type, *n* (%)	
Lobectomy	40 (44)
Pneumonectomy	46 (51)
Segmentectomy	4 (4)
Site, *n* (%)	
Left	43 (48)
Right	47 (52)

IGF1R Gene Copy Number

IGF1R gene copy number was evaluated using automated silver in situ hybridization (SISH), a chromogenic assay that allows for quantification of gene copy number concurrent to visualization of cell morphology in brightfield microscopy. The tissue microarray (TMA) containing triplicate cores per patient was probed according to the Ventana Medical Systems Inc. (Tucson, AZ) protocols for the INFORM IGF1R DNA probe. The probe was labeled with dinitrophenol and optimally formulated for use with ultraView SISH Detection Kit and accessory reagents on the Benchmark automated slide stainer. The IGF1R DNA probe was denatured at 95°C for 12 minutes, and hybridization was performed at 52°C for 4 hours. After hybridization, three stringency washes at 72°C were performed. The IGF1R DNA probe was visualized using a rabbit antidinitrophenol primary antibody and the ultraView SISH Detection Kit. The detection kit contained a goat antirabbit secondary antibody conjugated with horseradish peroxidase used as the chromogenic enzyme. The chemistry of the SISH reaction, briefly described, is driven by the sequential addition of silver A (silver acetate), silver B (hydroquinone), and silver C (H_2O_2). Here, the silver ions (Ag+) are reduced by hydroquinone to metallic silver atoms (Ag). This reaction is fueled by the substrate for horseradish peroxidase, hydrogen peroxide (silver C). The silver precipitation is deposited in the nuclei, and a single copy of the *IGF1R* gene can be visualized as a single discrete black dot, whereas a tight cluster of black dots stacked so closely together that individual signals cannot be resolved are considered amplified *IGF1R* genes. The specimen was then counterstained with Ventana's Hematoxylin II and Bluing reagent for interpretation by light microscopy. The number of *IGF1R* gene copies per nucleus was determined by two certified pathologists counting 50 nuclei, or less if the tissue microarray core was partially depleted, by two methods. In the first method, termed "focused," the pathologists scanned the core and focused on regions that appeared to have the highest copy numbers and counted 50 nonoverlapping nuclei that had the highest copy numbers. In the second method, termed "consecutive," the pathologists scanned the slide and found a region with high copy number and counted the signals in 50 consecutive nonoverlapping nuclei. Individual signals, black dots, were given a score of one, and if clusters were present, the numbers of signals within the cluster were estimated based on size of the cluster. The scores were analyzed to determine the mean of *IGF1R* gene copy number per nucleus.