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Original contribution

Insulin-like growth factor-1 receptor protein expression and gene copy number alterations in non–small cell lung carcinomas☆,☆☆

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Summary Insulin-like growth factor-1 receptor (IGF-1R) is a tyrosine kinase receptor implicated in the pathogenesis of several malignancies and is potentially an attractive target for anticancer treatment. In this study, we included 379 patients who underwent surgical resection (179 diagnosed as having adenocarcinoma [ADC]; 150, squamous cell carcinoma [SCC]; 41, sarcomatoid carcinoma and 9, large cell carcinoma). IGF-1R expression and gene copy number were assessed by immunohistochemistry and bright-field in situ hybridization (BISH), respectively. IGF-1R expression in non–small cell lung carcinoma was observed in 41.4% of samples and was more prevalent in SCC (69.3%) than in ADC (25.1%), large cell carcinoma (33.3%), and sarcomatoid carcinoma (12.2%) ($P < .001$). Among ADCs, most mucinous ADCs (75%) showed strong membranous staining with the IGF-1R antibody. Compared with protein expression, *IGF-1R* gene alteration was rare (8.4%). A statistically significant correlation between IGF-1R expression and positive *IGF-1R* BISH was observed ($\gamma = 0.762$, $P < .001$). IGF-1R–positive tumors were more common in smokers ($P = .004$), and these tumors were larger ($P = .006$) than the IGF-1R–negative tumors. *IGF-1R* BISH positivity was not correlated with any clinicopathologic factor. IGF-1R expression and *IGF-1R* BISH positivity were not correlated with overall survival. IGF-1R is highly expressed in SCC and mucinous ADC, although copy number alterations in the *IGF-1R* gene were rare. These findings may have important implications for future anti–IGF-1R therapeutic approaches.

1. Introduction

Normal cell growth is tightly regulated through the activation of cellular signal transduction pathways. Dysregulation of these pathways by overexpression or constitutive activation can promote tumor progression. Therefore, these pathways are considered therapeutic targets: one such

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example is trastuzumab (humanized monoclonal antibody for human epidermal receptor 2 [HER2]) for patients with breast cancer who have with HER2 overexpression [1]. Current recommendations include determination of HER2 status in all invasive breast cancers by immunohistochemistry (IHC) or fluorescence in situ hybridization [2]. Thus, careful measurement of biomarkers is necessary to identify a subset of patients having receptor-driven tumors.

The insulin-like growth factor (IGF) signaling pathway is a major regulator of fetal development, growth, and metabolism [3]. The central components of this signaling module are the IGF-1 receptor (IGF-1R), which is a glycosylated heterotetramer composed of 2 extracellular α and β subunits that have intrinsic tyrosine kinase activity. In malignant tumors, several model systems have provided evidence that the proliferative and metastatic potentials of cancer cells are enhanced by IGF-1R activation [4,5], suggesting that the potential therapeutic use of agents targeting this pathway may be broad.

For anticancer therapy targeting IGF-1R, approximately 25 compounds are currently in different stages of clinical and preclinical development [6]. In a phase II randomized trial, patients who received an antibody to IGF-1R (figitumumab) had a superior response rate. In particular, patients with pulmonary squamous cell carcinoma (SCC) showed a dramatic response [7]. To facilitate the development of IGF-IR inhibitors as therapeutics for cancer, identification of biomarkers for selecting patients who would be most likely to benefit clinically is needed. A recent in vitro study showed that expression of IGF-1R was correlated with sensitivity to IGF-1R inhibitors [8]. The IGF-1R expression measured by IHC ranged from 12.1% to 91.6% in non–small cell lung carcinoma (NSCLC) [5,9-13]. However, only 1 study has analyzed both IGF-1R protein expression and *IGF-1R* gene copy number changes in NSCLC [13].

In the present study, we analyzed the expression of IGF-1R in NSCLC by IHC using a recently developed rabbit monoclonal antibody and *IGF-1R* gene copy number alterations by bright-field in situ hybridization (BISH). We also analyzed the correlations between IGF-1R expression and *IGF-1R* gene copy number alterations, and clinicopathologic parameters and the status of epidermal growth factor receptor (*EGFR*) and *K-ras* mutations and *anaplastic lymphoma kinase* (*ALK*) rearrangements.

2. Materials and methods

2.1. Case Selection

The institutional review board of the National Cancer Center (Tokyo, Japan) approved this study. The specimens used in this study were obtained from 379 patients who underwent lung resection for adenocarcinoma (ADC), SCC, large cell carcinoma (LCC), or sarcomatoid carcinoma (SAC) at the National Cancer Center Hospital (Tokyo, Japan) between 1997 and 2007. Histologic diagnosis was based on the schema of the latest World Health Organization classification [14] and newly proposed ADC classification [15]. These cases were reclassified using 10 antibodies such as p63, high-molecular-weight cytokeratin, cytokeratin 5/6, Sox2, thrombomodulin, desmocollin-3, S100A7, S100A2, glypican-3, and thyroid transcription factor-1 [16]. Furthermore, all cases were subdivided into 3 differentiation grades, largely according to the Japanese Lung Cancer Society criteria. For ADC, well-differentiated tumors were consistent with predominant lepidic or papillary [17] patterns, moderately differentiated tumors were consistent with a predominant acinar pattern, and poorly differentiated tumors were consistent with a solid growth pattern. For SCC, well-differentiated tumors had an obvious stratified pattern, various uniform or slight pleomorphic nuclei, and significant keratinization; moderately differentiated tumors had a stratified pattern, although the degree of stratification and keratinization was lower than that of the well-differentiated tumors; and poorly differentiated tumors primarily exhibited solid growth and only had focal stratified patterns and keratinization.

We collected the patients' age, sex, smoking history, treatment modalities, outcome, maximum tumor size (cm), and pathological stage (p-stage). Staging was based on guidelines of the seventh edition of the TNM classification for lung cancer [18].

2.2. Microarray construction

The most representative tumor areas were sampled for tissue microarray (TMA) analysis. The TMAs were assembled with the tissue-arraying instrument KIN-1 (Azumaya, Tokyo, Japan). To reduce sampling bias caused by tumor heterogeneity, we used duplicate core samples measuring 2.0 mm in diameter taken from 2 different areas of each tumor.

2.3. Immunohistochemistry

The BenchMark XT automated slide processing system (Ventana, Tucson, AZ) was used according to the manufacturer's protocol for IGF-1R IHC staining. Briefly, TMA sections were incubated at 65°C for 20 minutes before the deparaffinization step with EZ Prep (Ventana) at 75°C for 16 minutes. Deparaffinized tissue sections were processed for a heat treatment using Cell Conditioning 1 solution (Ventana). The slides were incubated with a rabbit monoclonal anti–IGF-1R antibody (clone G11; Ventana) for 16 minutes at 37°C. IGF-1R protein was visualized with *Ultra*View DAB Universal Detection Kit (Ventana) followed by counterstaining with Hematoxylin II (Ventana) and Bluing Reagent (Ventana).