Clinical and Immunohistochemical Study on the Expression of Low-Density Lipoprotein Receptor-Related Protein-1 in Non-Small Cell Lung Cancers

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Directed by Professor Se-Kyu Kim

The Master's Thesis submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medicine

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I would like to dedicate this paper to all of you with all of my heart. Thanks everyone.

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ABSTRACT

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Background: Low-density lipoprotein receptor related protein-1 (LRP-1) is a multifunctional receptor involved in receptor-mediated endocytosis and cell signaling. It plays a role in modulating proteinase activity, which is necessary for tumor invasion. Although several studies showed that increased expression of LRP-1 in different types of cancer cells, its function remains unknown, especially in lung cancer. Therefore, this study aimed to investigate LRP-1 expression in non-small cell lung cancer (NCSLC) and

whether its expression is associated with pathological parameters and clinical features of non-small cell lung cancer.

Methods: One-hundred and five patients diagnosed as stage I NSCLC from 1995 to 2003 were included and immunohistochemical staining for LRP-1 was performed on the paraffin-embeded lung cancer tissue section. LRP-1 expression positive was defined as over 30% positive staining of the whole section. Clinical parameters were reviewed retrospectively and survival analyses were conducted according to LRP-1 expression in non-small cell lung cancer tissue.

Results: The mean age was 61.4 ± 9.33 years and male were 77.1%. The number of patients in stage IA and IB were 26 (24.8%) and 79 (75.2%) respectively. There were 3 different types of lung cancer on pathologic diagnosis; squamous cell carcinoma (n=52, 49.5%), adenocarcinoma (n=34, 32.4%) and others (n=19, 18.1%). Deaths occurred in 45 out of 105 patients and overall 5-year survival rate was 73.3%. Among them, 27 died of NSCLC. LRP-1 expression was positive in the stroma of 47 out of 105 NSCLC tissues (44.8%), whereas in the cancer cells of 7 out of 107 (6.7%). All the cancer cell LRP-1 expression positive tissues were also positive in the

stroma. There were no significant difference in stroma LRP-1 expression between histologic types (p=0.76). In addition, stroma LRP-1 expression did not affect overall and cancer-specific survival. However, disease-free survival time was significantly longer in patients with stroma LRP-1 (-) compared to those with stroma LRP (+) [117.8 (95% CI 93.4-140.5) vs. 145.2 (95% CI 128.5-161.9) months, p=0.04]. The 5-year disease-free survival rate was significantly higher in the stroma LRP-1 (-) group compared with the stroma LRP-1 (+) group (81.8% vs. 65.3%, p=0.04). In multivariate analyses adjusted for age, gender, smoking, tumor stage, and pathologic types of cancer, stroma LRP-1 (+) was identified as an independent predictor of recurrence (HR, 2.16; 95% CI, 1.13-4.12; p=0.02).

Conclusion: This study showed that LRP-1 was expressed in 44.7% of stroma surrounding stage I NSCLC and its expression is associated cancer recurrence suggesting that its LRP-1 may provide an important milieu for NSCLC migration and invasion. Further studies are required to elucidate LRP-1 function in lung cancer.

Key Words: Immunohistochemical stain, Low-density lipoprotein receptor related protein-1(LRP-1), Stage I Non-small cell lung cancer, stroma, milieu

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I. INTRODUCTION

Non small cell lung cancer (NSCLC) is the leading cause of death among all cancers worldwide although advances in therapeutic modalities have been made.[1, 2] In Korea, it is also the most common cause of death related to cancer.[3] In particular, 30 to 40% of the patients die of recurrence in spite of radical surgery to cure lung cancer.[1, 4]

Low-density lipoprotein receptor-related protein-1 (LRP-1) is a member of LDL receptor family and is expressed in hepatocyte, macrophage,

fibroblast, astrocyte, and placenta.[5] It is synthesized as a large transmembrane protein (molecular weight 600kDa) with two subunits of a 515-kDa α chain and an 85-kDa transmembrane β chain.[6, 7] It has been reported that LRP-1 regulates endocytosis and intracellular signal pathway through interaction with a variety of structurally diverse ligands.[6] The extracellular domain of LRP-1 recognizes more than 40 ligands and these LRP-1 bound ligands regulate the activity of proteinases in extracelluar matrix (ECM), thus are involved in lipid metabolism, cell growth, and tissue invasion.[5, 7] Besides a role as endocytic receptor, LRP-1 also regulates directly cell signaling pathway such as extracelluar signal regulated kinase (ERK)/mitogen-activated protein (MAP), phosphatidylinositol 3-kinase (PI3K), and c-Jun NH2-terminal protein kinase (JNK) in vascular wall, neuron, and lung.[8,9]

In addition, it has been suggested that LRP-1 plays a role in abnormal cell survival of cancer as a sensor of receptor function model in the process of carcinogenesis.[10-12] LRP-1 is expressed in glial cell tumor, low grade melanocytic tumor, and fibrosarcoma.[13-15] Valerie et al reported that LRP-1 expression was increased under hypoxic condition and the rate of metastasis to the lung was significantly decreased when LRP-1 was

silenced.[16] These results suggest that LRP-1 may facilitate the development and growth of cancer metastases in vivo.

To date, there have been few experimental reports on relationship between LRP-1 and lung cancer.[17] However, clinical studies regarding LRP-1 using human lung cancer tissue has not been reported yet. Therefore, this study aimed to investigate whether LRP-1 is expressed in human lung cancer and whether LRP-1 expression is associated with clinical features and prognosis in stage I non-small cell lung cancer (NSCLC).

II. MATERIALS AND METHODS

1. Study subjects

A total of 105 patients who underwent lung resection with curative intent from 1995 to 2003 at Severance hospital, Yonsei University Health System and Kangnam Severance hospital were enrolled. All patients had no lymph node metastasis and were diagnosed as stage I NSCLC. Tissue samples were collected after tumor resection. After fixed in buffered formalin tissue samples were embedded in paraffin block and 5 μm sections of paraffin embedded tissues were mounted in slides.

2. Immunohistochemistry

Slides were deparaffinized, hydrated in ethyl alcohol (100%, 90%, 70%, 50%), and washed in PBS water. Antigen retrieval was carried out in 10 mM sodium citrate buffer for 20 minutes using a Black and Decker vegetable steamer. For LRP-1, the primary monoclonal anti-LRP-1 antibody (LRP1 (5A6), Santa Cruz Biotechnology Inc, USA) was diluted in 1:100 blocking solution and was applied for 10 minutes incubation at humidified room air. After washing, a secondary goat anti-mouse IgG (Dako REAL™

EnVision™ Detection system, Dako Cytomation, Denmark) was added for 30 minutes, and the slides were then washed with PBS. Antigens were visualized with the avidin–biotin–peroxidase complex after 5 min of incubation with the chromogen diaminobenzidine (DAB) as a co-substrate. Finally, sections were lightly counterstained with hematoxylin and mounted with xylene-based DPX mounting medium.

Normal placental tissue and omission of primary antibody served as a positive and negative control, respectively.

Immunostaining was assessed semiquantitatively as the percentage of positive staining of the whole section according to the following scale: score 0, 0 to 10 % staining; score +1, 10 to 30 % staining; score +2, 30 to 50 % staining; score +3, > 50 % staining. LRP-1 stain positive was defined as more than +2 staining. The sections were examined and scored by two independent observers, then reviewed together, and the average data represent a consensus value of all observations.

3. Clinical data collection

Demographic and clinical data were retrospectively reviewed with patients' medical records; age, gender, smoking, tumor stage, pathologic diagnosis,

recurrence, metastasis, and death. Study endpoint was set at January 31, 2009. This study was approved by the institutional review board for human research at Yonsei University College of Medicine, Kangnam Severance Hospital.

4. Statistical analysis

Statistical analysis was performed using SPSS for Windows software, version 12.0 (SPSS Inc., Chicago, Illinois, USA). All data were expressed as mean±SD or range with median for the skewed data. Kolmogorov-Smirnov test was used to analyze the normality of the distribution of the parameters measured. The comparisons between LRP-1 positive and negative groups were made by chi-square test and Student's *t*-test for normally distributed variables and Mann-Whitney U-test for skewed variables. Patient overall, disease-free, and cancer-specific survival rates were determined by the Kaplan–Meier method. For cancer-specific survival analysis, deaths unrelated to cancer were treated as censored. A log-rank test was used to compare survival rates between the two groups. A multivariate Cox proportional hazards model was utilized to identify factors predicting patient mortality and to estimate and test the hazard ratio (HR) and associated 95%

confidence intervals (CI). All probabilities were two-tailed and the level of significance was set at 0.05.

III. RESULTS

1. Patient characteristics

The mean age of the patients was 61.4 ± 9.33 (31~83) years and male were 77.1%. Current and ex smokers were 57.1% and 7.6% respectively and the remaining was non-smokers (35.2%). The median smoking amount was 30 ± 2.8 (8~100) pack-years. All patients were surgically treated with lobectomy (n=81, 77.1%), pneumonectomy (n=22, 21.0%), and wedge resection (n=2, 1.9%). There were 3 different types of lung cancer on pathologic diagnosis; squamous cell carcinoma (n=52, 49.5%), adenocarcinoma (n=34, 32.4%) and others (n=19, 18.1%). Of the 105 patients, 26 (24.8%) and 79 (75.2%) patients were at stage IA and IB, respectively. The median follow-up duration after surgery was 119.7 ± 5.48 (4.9~198.6) months (Table 1).

Table 1. Clinical characteristics of patients

Number of patients	105
Age (mean, range)	61.4 ± 9.33 (31 ~ 83)
Sex (male:female)	81 : 24
Smoking status	
Current smoker	60 (57.1%)
Non-smoker	37 (35.2%)
Ex-smoker	8 (7.6%)
Smoking amount	30 ± 2.8(8-100) pack-years*
Histology	
Squamous cell carcinoma	52 (49.5%)
Adenocarcinoma	34 (32.4%)
Others	19 (18.1%)
Stage	
1A	26 (24.8%)
1B	79 (75.2%)
Operation	
Lobectomy	81 (77.1%)
Pneumonectomy	22 (21.0%)
Wedge resection	2 (1.9%)
Recurrence/metastasis	
Disease free	67 (63.8%)
Recurrence/metastasis	38 (36.2%)

* smoking amount is skewed data. Data is expressed as median ±SE with range.

2. LRP-1 expression in normal and tumor tissue

Immunohistochemical analysis showed that LRP-1 immunoreactivity was not observed in negative control group (omission of primary antibody) whereas it was strongly observed in positive control group (placenta tissue). Particularly, it was highly expressed in cytoplasm of syncytial trophoblast and along villi in placenta tissue. However, LRP-1 was not expressed in fetal capillary (Figure 1A).

In normal lung tissue beside of the tumor, LRP-1 expression was not observed in ciliated bronchial epithelial cells and type 1 alveolar cells. It was faintly expressed in interstitial macrophages with less than 10% (Figure 1B).

Of the 105 patients, 47 had more than +2 positive staining of LRP-1 expression in the stroma (Figure 1E, 1F). In contrast, cancer cell LRP-1 expression was observed in only 7 patients of the 47 stroma LRP-1 positive patients(Figure 1C, 1D) (Table2). In the resting 58 patients, stroma and cancer cell LRP-1 expression was not observed or faintly observed without significance.

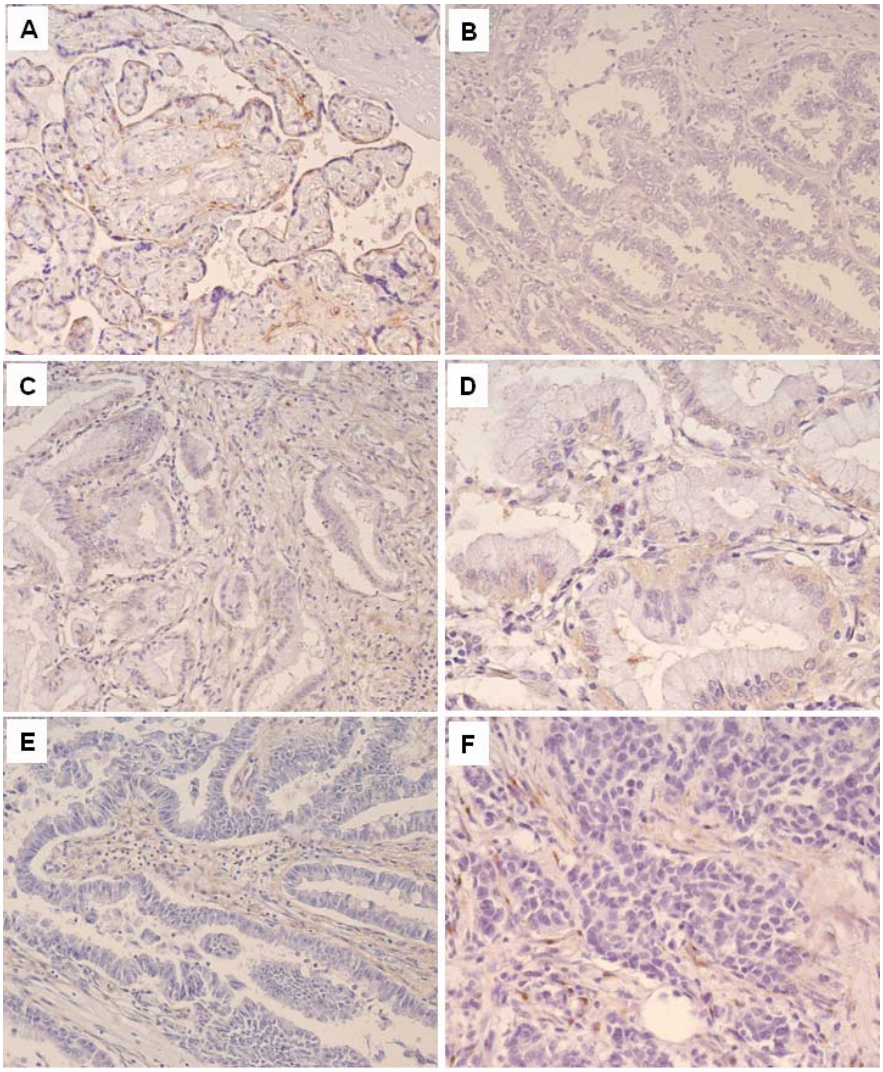

Figure 1. Immunohistochemical staining for LRP-1 in NSCLC tissues. The positive LRP-1 stained placenta control (A, x200) and negative stained control (B, x200). The positive LRP-1 staining was seen within cancer cells (C, x200, D, x400) and within stroma (E, x200, F, x400).

Table 2. LRP-1 expressions characteristics in non-small cell lung cancer tissue

Histologic site	LRP-1 expressions (%)					
	0*	1+†	2+‡	3+§	Negative	Positive
Cancer cell	70 (66.7)	28 (26.7)	6 (5.6)	1 (1.0)	98 (93.3)	7 (6.7)
Stroma	48 (45.8)	10 (9.5)	32 (30.4)	15 (14.3)	58 (55.2)	47 (44.8)

Semiquantitative immunostaining score as the percentage of positive staining of the whole section

* score 0 : 0 to 10 % staining of the whole section

† score +1 : 10 to 30 % staining of the whole section

‡ score +2 : 30 to 50 % staining of the whole section

§ score +3 : > 50 % staining of the whole section

3. Relationship between LRP-1 expression and clinical parameters

Patients were classified into 2 groups according to stroma LRP-1 expression and demographic and clinical parameters were compared. There were no significant differences in all parameters between stroma LRP-1 expression positive and negative group. Compared to the stroma LRP-1 expression positive group, age, gender, and smoking status were not different in the stroma LRP-1 expression negative group. Disease free survival rate was lower in the stroma LRP-1 expression positive group than in the stroma LRP-1 expression negative group, but it did not reach statistical significance (57.4% vs. 69.0%, p=0.22) (Table 3).

We conducted the same comparative analyses between cancer cell LRP-1 expression positive and negative groups. There were no significant differences in age, gender, smoking status, and disease free survival rate between the 2 groups (Table 3).

Table 3. Comparison of demographic and clinical parameters among stroma LRP-1 expression (+) / (-) groups and cancer cell expression LRP-1 (+) / (-) groups

Characteristics (n)	Expression of LRP-1					
	Stroma (-) (n=58)	Stroma (+) (n=47)	*p*	Cell (-) (n=98)	Cell (+) (n=7)	*p*
Gender						
Male (81)	44 (75.9%)	37 (78.7%)	0.73	75 (76.5%)	6 (85.7%)	0.58
Female (24)	14 (24.1%)	10 (21.3%)		23 (23.5%)	1 (14.3%)	
Age (years)						
< 60 (42)	26 (44.8%)	16 (34.0%)	0.26	40 (40.8%)	2 (28.6%)	0.52
≥ 60 (63)	32 (55.2%)	31 (66.0%)		58 (59.2%)	5 (71.4%)	
Smoking status						
Nonsmoker (37)	18 (31.0%)	19 (40.4%)	0.32	33 (33.7%)	4 (57.1%)	0.21
Current and ex-smoker (68)	40 (69.0%)	28 (59.6%)		57 (58.2%)	3 (42.9%)	
Disease free survival						
Disease free (67)	40 (69.0%)	27 (57.4%)	0.22	62 (63.3%)	5 (71.4%)	0.66
Recurrence/mets (38)	18 (31.0%)	20 (42.6%)		36 (36.7%)	2 (28.6%)	

4. LRP-1 expression according to pathologic types of NSCLC and staging

Stroma LRP-1 expression was observed in 22 cases with squamous cell carcinoma (46.8%), 17 with adenocarcinoma (36.1%), 8 with other carcinoma (17.1%). There was no significant difference in stroma LRP-1 expression among pathologic types of NSCLC (p=0.76, Table 5). In addition, cancer staging was not different between stroma LRP-1 expression positive and negative groups (p=0.77, Table 5).

Compared to cancer cell LRP-1 expression negative group, pathologic types and cancer staging were also similar in cancer cell LRP-1 expression positive group (Table 6).

Table 4. Stroma LRP-1 expression and cancer cell LRP-1 expression according to pathologic types of NSCLC and staging

Characteristics (n)	Expression of LRP-1					
	Stroma (-) (n=58)	Stroma (+) (n=47)	*p*	Cell (-) (n=98)	Cell (+) (n=7)	*p*
Cell type						
Squamous cell (52)	30 (51.7%)	22 (46.8%)		49 (50.0%)	3 (42.8%)	
Adenocarcinoma (34)	17 (29.3%)	17 (36.1%)	0.76	32 (32.7%)	2 (28.6%)	0.76
Others (19)	11 (19.0%)	8 (17.1%)		17 (17.3%)	2 (28.6%)	
Stage						
1A (26)	15 (25.9%)	11 (23.4%)		24 (24.9%)	2 (28.6%)	
			0.77			0.81
1B (79)	43 (74.1%)	36 (76.6%)		74 (75.1%)	5 (71.4%)	

5. Survival analyses according to LRP-1 expression

The median follow-up duration after surgery was 119.7 ± 5.48 (4.9~198.6) months. All-cause deaths occurred in 45 patients (42.9%), among whom 27 (25.7%) died of lung cancer. In addition, of the 38 patients (36.1%) who underwent recurrence or metastasis, 27 died of NSCLC (78.4%).

The overall 5-year survival rate was 73.3% and it was not significantly different between stroma LRP-1 expression positive and negative groups (68.1% vs. 77.6%, p=0.11) (Figure 2). The mean survival duration was 119.4 [95% confidence interval (CI), 97.7~141.1] months in the stroma LRP-1 expression positive group whereas it was 139.6 (95% CI, 122.7~156.5) months in the stroma LRP-1 expression negative group. In contrast, the 5-year disease-free survival rate was significantly higher in the stroma LRP-1 expression negative group compared with the stroma LRP-1 expression positive group (81.8% vs. 65.3%, p=0.04) (Figure 3). The mean disease-free duration was significantly longer in the stroma LRP-1 expression negative group than in the stroma LRP-1 expression positive group [145.2 (95% CI, 128.5~161.9) vs. 117.0 (95% CI, 93.4~140.5) months, p=0.04].

After 22 deaths not related to lung cancer were treated as censored, we

calculated lung cancer-specific survival rate in the remaining patients. Compared to the stroma LRP-1 expression positive group, the 5-year lung cancer-specific survival rate was higher in the stroma LRP-1 expression negative group (80.4% vs. 60.5%) (Figure 4). However, it did not reach statistical significance (p=0.11). The mean lung cancer-specific survival duration was 161.6 (95% CI, 146.0~177.3) and 144.5 (95% CI, 122.3~166.8) months in the stroma LRP-1 expression negative and stroma LRP-1 expression positive group, respectively.

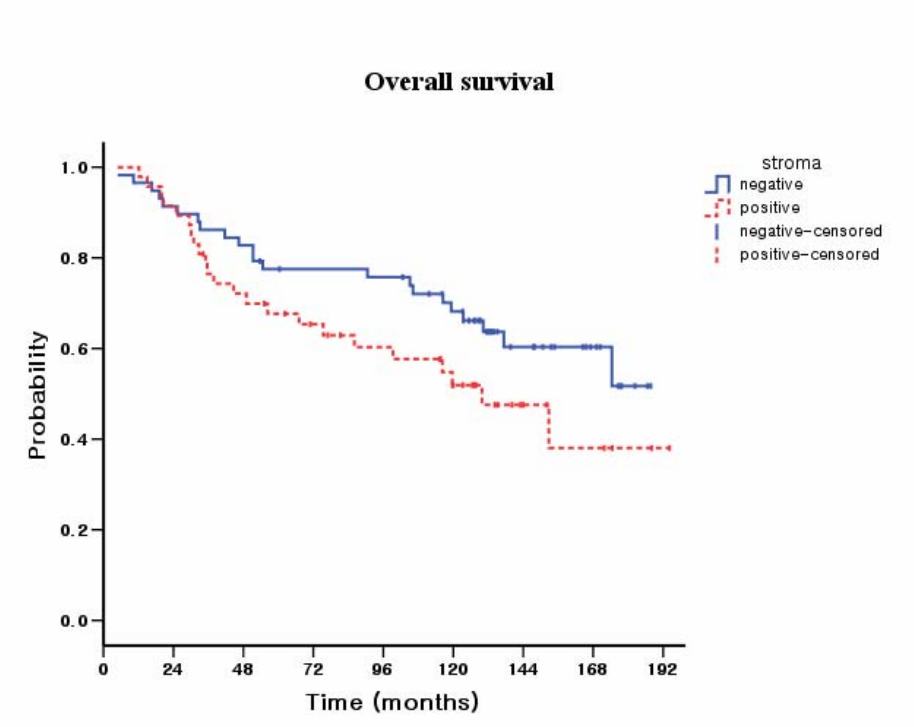

Figure 2. Overall Kaplan-Meier survival curve according to stroma LRP-1 expression in stage I NSCLC. There was no significant difference between the two groups (p=0.11).

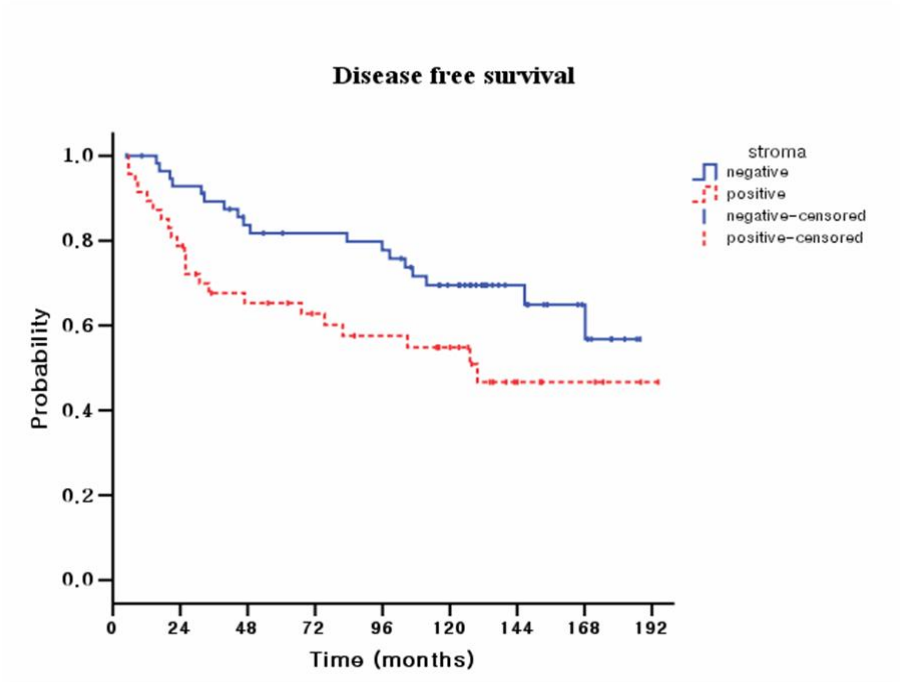

Figure 3. Disease free survival curve according to stroma LRP-1 expression in stage I NSCLC. The mean disease-free survival duration was significantly longer in the stroma LRP-1 expression negative group [145.2 (95% CI, 128.5~161.9) vs. 117.0 (95% CI, 93.4~140.5) months, p=0.04].

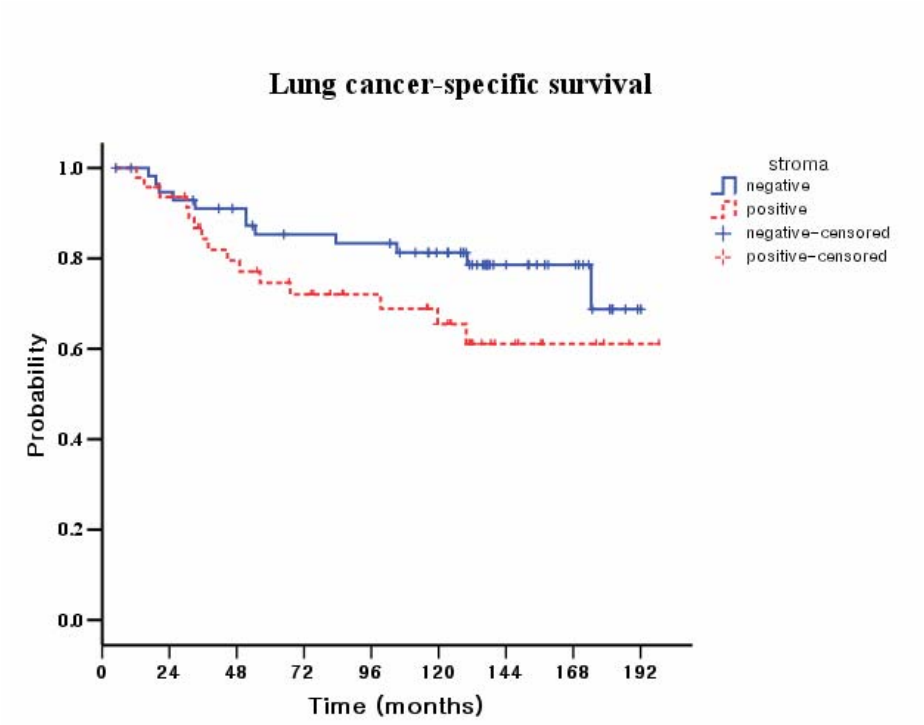

Figure 4. Lung cancer-specific survival curve according to stroma LRP-1 expression in stage I NSCLC. There was no significant difference between the two groups (p=0.11).

6. Multivariate Cox regression analyses for overall mortality, recurrence, and cancer-specific mortality

To determine overall mortality, recurrence, and cancer-specific mortality, we performed multivariate Cox regression analyses adjusted for age, gender, smoking, pathologic types, and stage. For overall mortality, stroma LRP-1 expression was not a significant predictor of death [hazard ratio (HR), 1.62; 95% confidence interval (CI), 0.90-2.93; P=0.10] (Table 5). However, it was an independent predictor of recurrence (HR, 2.16; 95% CI, 1.13-4.12; p=0.02) (Table 6). Finally, stroma LRP-1 expression was not a significant determinant of cancer-specific mortality (HR, 1.80; 95% CI, 0.98-3.31; p=0.06) (Table 7).

Table 5. Multivariate Cox regression analyses for overall mortality

	Hazard ratio	95% CI	*p*
Stroma LRP-1 (+) [vs. LRP-1(-)]	1.62	0.90-2.93	0.10
Sex (vs. male)	1.18	0.41-3.38	0.76
Age (per 1-year increase)	1.02	0.98-1.06	0.39
Smoking			
No	Reference		
Current and ex-smoker	2.25	0.88-5.80	0.09
Stage (vs. IA)	1.44	0.65-3.20	0.37
Pathology			
Squamous cell carcinoma	Reference		
Adenocarcinoma	1.30	0.66-2.56	0.44
Others	1.11	0.44-2.78	0.83

Table 6. Multivariate Cox regression analyses for recurrence

	Hazard ratio	95% CI	*p*
Stroma LRP-1 (+) [vs. LRP-1 (-)]	2.16	1.13-4.12	0.02
Sex (vs. male)	1.76	0.65-4.80	0.27
Age (per 1-year increase)	1.00	0.96-1.04	0.95
Smoking			
No	Reference		
Current and ex-smoker	2.72	1.03-7.20	0.04
Stage (vs. IA)	1.43	0.64-3.17	0.38
Pathology			
Squamous cell carcinoma	Reference		
Adenocarcinoma	2.12	1.01-4.46	0.05
Others	2.75	1.15-6.62	0.02

Table 7. Multivariate Cox regression analyses for cancer-specific mortality

	Hazard ratio	95% CI	*p*
Stroma LRP-1 (+) [vs. LRP-1 (-)]	1.80	0.98-3.31	0.06
Sex (vs. male)	1.68	0.58-4.90	0.34
Age (per 1-year increase)	1.01	0.97-1.05	0.57
Smoking			
No	Reference		
Ex-smoker	2.58	0.94-7.10	0.07
Stage (vs. IA)	2.01	0.83-4.89	0.12
Pathology			
Squamous cell carcinoma	Reference		
Adenocarcinoma	1.37	0.69-2.73	0.37
Others	1.19	0.47-3.01	0.72

IV. DISCUSSION

Low density lipoprotein (LDL) receptor-related protein 1 (LRP-1) is a member of LDL receptor and serves as endocytic receptor. LRP-1 is involved in various biologic function through ligand interaction with lipoprotein, growth factor, matrix macromolecules, proteinase, and proteinase inhibitor complex.[18] In particular, LRP-1 dependent endocytosis is reported to be a key mechanism in ECM degradation by activation of proteinases such as matrix metalloproteinase, thus mediate tumor cell invasion and growth.[19] This finding suggests that LRP-1 may be a novel therapeutic target in cancer.

Cancer cells including lung cancer, which are characterized by rapid growth, require angiogenesis for cell proliferation and tumor growth.[20, 21] Hypoxia is one of the strong stimuli for angiogenesis.[22] Although hypoxia-inducible factor-1α (HIF-1α) under hypoxic conditions is presumed to play a role in cancer cell growth,[23] its underlying mechanism responsible for tumor growth remains elusive. Lialo et al reported that conditional deletion of HIF-1α gene retarded tumor growth and decreased pulmonary metastasis in a transgenic mouse model for metastatic breast cancer,[24] suggesting that

hypoxia-induced genes may be involved in the process of cancer initiation, progression, and metastasis and may also be a therapeutic target.[25] In this regard, Koong et al reported that hypoxia increased the expression of LRP-1 mRNA in various cancer cells,[26] indicating that LRP-1 induced by hypoxia may be implicated in tumor growth.

In invasive cell clones derived from human prostate and breast tumor cells, the expression of LRP-1/α-2 macroglobulin receptor is decreased.[27] In line with this finding, LRP-1 expression is lower in Wilm's tumor, endometrial cancer, and thyroid cancer.[28-30] In contrast, Li et al reported that in vitro invasiveness of human breast cancer cells was promoted by LRP.[31] In addition, LRP-1 silencing prevents malignant cell invasion and migration in thyroid follicular carcinoma[32] and glioblastoma cell lines.[33] The mechanism for such discrepancy is unknown. It is possible that LRP-1 function varies depending on different ligands bound to LRP-1 in various cell types and ECM.

To date, there have been few studies on LRP-1 in lung cancer. Yamamoto et al reported that LRP-1 was expressed in 1 of 5 cases (20%) with pulmonary adenocarcinoma cell line,[13] but its clinical implication is currently unknown.

To our knowledge, this is the first report to investigate LRP-1 expression in human lung cancer tissue and our study includes the largest number of patients with lung cancer. In this study, stroma and cancer cell LRP-1 expression was observed in all types of lung cancer. It was most highly expressed in squamous cell carcinoma (46.8%), but its expression was not statistically significant among types. It is uncertain why LRP-1 expression is not favored in specific type of lung cancer. It is possible that LRP-1 is not involved in the development of cancer but in the process of tumor growth, migration, and invasion, thus LRP-1 expression is not affected by types of cancer cell per se.

It should be noted that LRP-1 was expressed in mostly in the stroma in our study. This finding contradicts the results from previous studies showing that it is highly expressed within cancer cell.[13-15] It can be speculated that cancer cell LRP-1 expression is lower than stroma LRP-1 expression due to lack of LRP-1-mediated lipid metabolism which is greatly involved in cell metabolism whereas it is increased in the stroma where migration and invasion of cancer cells are activated. Consistent with this speculation, LRP-1 is not expressed in normal lung tissue where lipid metabolism and ECM activity are not clearly evident. As LRP-1 is a transmembrane protein, it

mediates endocytosis on the cell surface through interaction with other signaling proteins.[9,18] In addition, LRP-1 serves as a crucial regulator of extraceullar proteolytic activity. It mediates tumor cell migration and invasion by inhibiting degradation of urokinase plasminogen activator (uPA) through receptor binding.[30, 34] Taken together, these findings suggest that LRP-1 does not appear to play a role in the initiation of cancer within cells but in the process of tumor growth, migration, and invasion in the stroma after cancer is already formed.

In this study, clinical parameters such as age, gender, and smoking were not associated with stroma and cancer cell LRP-1 expression. Interestingly, disease-free survival was significantly longer in the stroma LRP-1 expression negative group. In addition, stroma LRP-1 expression was an independent predictor of recurrence in the multivariate analysis (Table 8). This finding suggests that lung cancer may recur earlier in patients with stroma LRP-1 expression. In contrast, overall patient survival and cancer-specific survival were not significantly different according to stroma LRP-1 expression. This may be partly explained by the fact that staging in the study subjects is limited to IA and IB.

Several shortcomings should be discussed in this study. Because we

performed immunohistochemical staining to detect LRP-1 expression in human lung cancer tissue, quantitative assessment was not made. This study is retrospective in nature and we could not obtain adequate amount of tissue sample, thus semi-quantitative analysis such as Western blotting was not possible. In addition, this study was limited to patients with stage I. Tumor invasion and metastasis is not so frequent in this stage. Therefore, it would be interesting to investigate LRP-1 expression in a larger number of patients with more advanced stage. Finally, the mechanism responsible for the higher expression of LRP-1 in the stroma than in the cells should be further explored.

V. CONCLUSION

Stroma LRP-1 expression was observed in 44.7% of patients with stage I NSCLC. It was highly expressed in the stroma suggesting that its function may be more important in the process of tumor migration and invasion. However, Stroma and cancer cell LRP-1 expression was not associated with clinical parameters or pathologic types of NSCLC. Although stroma LRP-1 expression did not predict all-cause and cancer-specific mortality, it was identified as an independent predictor of recurrence. Further studies are required to elucidate LRP-1 function in NSCLC.

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ABSTRACT (in Korean)

비소세포폐암 환자의 임상 양상에 따른 Low-Density Lipoprotein Receptor-Related Protein-1 발현에 관한 면역조직화학적 조사

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정 재 희

LDL receptor protein-1(LRP-1)는 지질 대사 이외에 단백분해효소 활성을 조절하여 세포 전이 및 침투를 촉진시키는 것으로 알려져 있고, 유방암, 신경세포종, 흑색종 등에서 발현 시 악성화가 증가됨이 보고되었으나 폐암과의 연관에 대한 연구가 부족하였다.

이에 필자는 LRP-1 이 비소세포폐암 세포 및 조직에 분포하는 양상을 확인하고, 이의 발현이 비소세포폐암 환자에서 임상적 의의를 갖는지 알아 보고자 하였다.

1995년부터 2003년까지 1기 비소세포폐암의 진단 하에 완치 목적으로 폐절제술을 받은 105명의 환자들에서 얻어진 조직절편으로 LRP-1 항체를 이용하여 면역조직화학 염색을 시행하고 염색유무에

따른 임상 특징과 생존 기간의 차이에 대해 알아보았다.

본 연구에서 대상 환자들의 평균 연령은 61.4±9.33세이었고, 남자는 81명(77.1%)이었다. 진단 시 병기는 1A 26 예(24.8%), 1B 79 예(75.2%)이었고, 편평상피암 52예(49.5%), 선암 34예(32.4%), 기타 19예(18.1%)이었다. 5년 생존율은 73.3%이었으며, 총 사망 45예 중 27예는 폐암 재발로 사망하였다, 폐암세포 내에서 LRP-1의 발현을 보인 증례는 7예(6.7%%)였으며, 암세포 주변의 간질 조직에서 발현을 보인 경우는 47예(44.8%)였다. 성별, 나이, 흡연유무에 따른 LRP-1 발현의 차이는 없었고, 조직 유형은 편평상피암 22예(46.8%), 선암 17예(36.1%), 기타 암 8예(17.1%)에서 간질 조직 내 LRP-1 발현을 보였으나 각 군간 차이는 없었다(p=0.76). 간질에서의 LRP-1의 발현에 따른 5년 생존율은 발현군, 미발현군 각각 68.1%, 77.6%로 유의한 차이는 없었고(p=0.26), 평균 무병 생존기간은 각각 117.0개월(95% CI 93.4~140.5개월), 145.2개월(95% CI 128.5~161.9개월)로 LRP-1 미발현군에서 길었다(p=0.04). 간질에서의 LRP-1의 발현에 따른 5년 무병 생존율은 미발현군, 발현군에서 각각 81.8%, 65.3%로 LRP-1 미발현군에서 높았다(p=0.04). 나이, 흡연유무, 병기, 조직유형을 보정한 다중 분석에서 간질에서의 LRP-1 발현은 재발의 독립적 예측인자로 보였으나(HR, 2.16;

95% CI, 1.13-4.12; p=0.02) 생존 예측인자로서의 의미는 없었다.

결론적으로 1기 폐암 조직에서 LRP-1의 발현이 44.7%에서 관찰되었고, 세포간질에서 발현이 높았으며, 조직유형이나 임상 인자와의 관련성은 없었고, 수술 후 재발을 촉진시키는 경향을 보였다. 이러한 결과를 토대로 LRP-1은 폐암과의 연관성은 있으며, 암의 발생보다는 진행과 이동에 더 많이 관여할 것으로 생각된다.

핵심되는 말: 면역조직화학염색, LDL receptor protein-1, 1기 비소세포폐암, 세포간질