学位論文内容の要旨

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学 位 論 文 題 名

Down-regulated expression of human leukocyte antigen class I heavy chains is linked to poor prognosis in non-small cell lung cancer.

（非小細胞肺癌において HLA class I H 鎖の発現低下は予後不良と相関している）

Introduction and Purpose: The most effective treatment for early stage non-small-cell lung cancer (NSCLC) is surgical resection. However, over 60% of NSCLC patients relapse after surgery[1-3]. A classification system based on immunostaining for loss or down-regulation of HLA class I has not been established. In this study, we assessed the influence of down-regulation of HLA class I expression in tumors on the survival of patients with NSCLC using EMR8-5 immunostaining, SCID mice xenografts as quantitative controls, and new classification schemes based on tumor heterogeneity, and reviewed the correlation between loss or down-regulation of HLA class I expression and NSCLC prognosis.

Materials and methods: Immunohistochemical (IHC) stainings for HLA class I and β_2-microglobulin were performed on specimens from 111 patients with NSCLC. Surgical specimens were obtained from 72 men and 39 women with a mean age of 62.7 years (range 36-80 years). Median duration of follow-up was 60.9 months (range 4.0-100.7 months), and 43 patients (38.7%) died during the follow-up period. Our results were correlated with the patients' clinical records. A single section from deep within the tumor specimen was selected for analysis, and resected specimens were examined histopathologically following staining with hematoxylin and eosin. The overall survival curves were compared using the log-rank test. Multivariate analyses were carried out by Cox's proportional hazard model.

Results: Immunostaining results were grouped into three categories: strongly positive (equivalent to staining of alveolar epithelium); weakly positive relative to alveolar epithelium; and absent (no detectable staining). The cases were further divided into five categories: homogeneous strong staining, heterogeneous strong-weak staining, homogeneous weak staining, heterogeneous strong-weak-absent staining, and heterogeneous weak-absent staining. Strong staining was defined by the positive control and stages were graded in 10% steps. The cases were divided into five classes based on expression of HLA class I heavy chain and β_2-microglobulin. Our present data may reflect the result of emerging immune-escaping variants reflecting intratumor heterogeneity for HLA class I and β_2-microglobulin. Overall survival for patients with tumors lacking HLA class I heavy chain (30 cases; 27.0%) revealed a significant unfavorable. Multivariate analysis revealed the absence of HLA

class I heavy chain to be an independent factor of poor prognosis. Absence of β_2-microglobulin (58 cases; 52.3%) might tend to unfavorable prognosis. According to χ^2 testing, down-regulation of HLA class I heavy chain expression significantly correlated with down-regulation of β_2-microglobulin expression ($p<0.01$). Cases lacking both HLA class I heavy chain and β_2-microglobulin (23 cases; 20.7%) showed statistically significant unfavorable prognosis compared with other cases ($p<0.05$). The overall prognosis for cases lacking β_2-microglobulin was not significant. When divided by cancer stage, stage I-II cases β_2-microglobulin were not significant, neither.

Discussion: The aim of this study was to use to develop a quantitative immunohistochemistry protocol to investigate whether expression of HLA class I in NSCLC correlates with patient survival. We developed a reproducible and carefully controlled quantitative immunohistochemistry methodology that provided a robust approach to accurately classify NSCLC specimens into categories based on HLA class I expression levels and characteristics. One of the most important issues in past studies was a lack of consideration of tumor heterogeneity in IHC evaluation. Therefore, in this study, we classified HLA Class I down regulation in consideration of tumor heterogeneity assessed using EMR8-5 and SCID mice xenografts. Our methodology may be useful for others working in the field, and may provide a means to improve the consistency and comparability of similar studies in the future. We found that absence of HLA class I expression on tumors was an independent factor predicting poor prognosis, although expression of HLA class I heavy chain did not correlate with pathological variables. These results suggest that NSCLC would progress regardless of the expression of HLA class I heavy chain, and host immunity plays a limited role in influencing tumor growth prior to surgery. However, after surgery, more cases lacking HLA class I relapsed than cases with HLA class I expression. Moreover, the prognosis was good for early stage HLA class I heavy chain-positive cases, although in later stage cases there was no correlation between prognosis and expression of HLA class I heavy chain consistent with previous studies. Interestingly, immunologic responses were independent of stage and prior therapy in a study of NSCLC dendritic cell vaccines, consistent with the possibility that the effectiveness of immunotherapy may be related to expression of HLA class I heavy chains on tumor cells. Currently, recurrent or surgically non-resectable cases are often treated with immunotherapy, even though the patients' immune systems have a limited capacity to deal with the heavy tumor burden. Our data suggest that immunotherapy would be beneficial as postoperative adjuvant therapy in NSCLC.

Conclusions: Our data demonstrate that an absence of HLA class I heavy chain in tumor cells is an independent prognostic factor for NSCLC and would play an important role in the immune surveillance of patients.