Title: Expression of phosphatase and tensin homolog and programmed cell death ligand 1 in adenosquamous carcinoma of the lung

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Introduction
Lung adenosquamous cell carcinoma (ASC) is a rare variant of non-small cell lung cancer (NSCLC) with poor prognosis than the more common variants, adenocarcinoma (ADC) and squamous cell carcinoma (SCC), and more aggressive. Certain biological differences may exist between these three histological types of NSCLC. Tyrosine kinase activation contributes to tumor growth and malignant progression in different tissues, including lung cancer. The phosphoinositide 3-kinase (PI3K) signaling axis plays important regulatory roles in cell proliferation and differentiation by activating several oncogenes and downstream effector pathways causing tumorigenesis. It can be activated by the loss of phosphatase and tensin homolog (PTEN), mutations in the genes encoding PI3K, or constitutive activation of upstream regulatory receptor tyrosine kinase pathways. In various cancer types, it has been suggested that programmed cell death ligand 1 (PD-L1) expression can be induced by innate immune resistance including PTEN loss and adaptive immune resistance, the alternative mechanism. Interactions between programmed cell death 1 (PD1) and its ligand (PD-L1) constitute a key immune checkpoint, which maintain self-tolerance and protect peripheral tissues by modulating immune responses. Ligation of PD-L1 on cancer cells to PD1 on T cells suppresses T cell activation and proliferation, inducing T cell apoptosis. Little is known about the relationship between the expression patterns of PD-L1 and PTEN in ASC. To better understand the biological mechanism underlying the immune and oncogenic nature of ASC, we investigated PD-L1 and PTEN expression levels in ASC, ADC, and SCC, using clinicopathological data.

Materials and Methods
Among the collected samples, some were excluded because of the absence of tumor cells, presence of questionable inflammatory cells, or edge artifacts in immunohistochemistry (IHC). Therefore, for PD-L1 IHC staining, 28 cases of ASC, 133 cases of ADC, and 88 cases of SCC were included. For PTEN IHC staining, 27 cases of ASC, 148 cases of ADC and 102 cases of SCC were scored. For ASC, full-section hematoxylin and eosin-stained slides were reviewed for both squamous and glandular components in at least 10% of the tumor area based on the World Health Organization criteria. In cases of histologically atypical
adenocarcinoma, IHC analyses for napsin A, thyroid transcription factor 1, and for the atypical squamous components, p40 and CK14 were also employed to confirm the final results. For ADC and SCC, tissue microarray slides containing 0.6mm cores of lung ADC and SCC cases were used. IIIC analyses of 4-μm thick tissue sections stained with anti-PD-L1 monoclonal antibody (clone 28-8) and an anti-PTEN antibody (clone D4.3) were carried out using Ventana Bench Mark XT Automated stainer (Ventana Medical Systems, Tucson, AZ, USA) and BOND III fully automated stainer (Leica Biosystems, Melbourne, Australia) following standard protocols. After staining, all slides were scanned, scored and analyzed for the expression of PTEN and PD-L1. In ASC, the expression of squamous cell component and adenocarcinoma component were analysed separately. Chi-square and Fisher’s test will be used to calculate the $p$ value. If the $p$ value is less than 0.05 ($p<0.05$), it is considered statistically significant.

**Results**

PD-L1 expression was similar between the adenocarcinoma component of ASC vs. lung ADC and between the squamous component of ASC vs. lung SCC. PTEN loss was higher in lung ADC than in the adenocarcinoma component of ASC and significantly higher in lung SCC than in the squamous component of ASC. PD-L1 expression was higher in the squamous component than in the glandular component of the 28 ASC cases, but PTEN loss was similar. Overall, PTEN loss was higher in lung SCC than in lung ADC and both components of ASC. In lung SCC and glandular portions of ASC, PD-L1 expression levels were significantly associated with those of PTEN. The loss of PTEN correlated with smoking status in patients with lung ADC.

**Discussion**

Our results implied that both squamous and glandular components of ASC may share the same oncogenic driver pathway for carcinogenesis. However, the squamous cell components of ASC likely escape the immune surveillance better than the glandular components due to higher PD-L1 expression. It has been suggested that lung ADC can progressively transdifferentiate to SCC with pathologically mixed ASC representing the intermediate stage, which might confer drug resistance and worse prognosis. Therefore, the gain of PD-L1 expression during phenotype transdifferentiation from adenocarcinoma to squamous cell carcinoma in lung ASC may help tumor cells to escape immune surveillance, consequently resulting in poor prognosis. We identified that PTEN loss was greater in lung SCC than in lung ADC which was similar to other reports. Furthermore, PTEN loss was more significant in SCC than in the squamous component of ASC. Therefore, the biology of lung ASC may be more complex and divergent than that of SCC. PD-L1 expression levels were similar between the squamous cell component of ASC and lung SCC, as well as between the glandular component of ASC and lung ADC. Therefore, no trend was apparent for differential PD-L1 expression between SCC or ADC and ASC. This finding also suggests that anti-PD-1/PD-L1 monoclonal antibodies are a promising therapeutic strategy for ASC patients with PD-L1 upregulation. In SCC, 80% of PD-L1-negative cases showed PTEN loss. It may be deduced that lung SCC characterized by the loss of the tumor suppressor PTEN can proceed to tumorigenesis without PD-L1 expression. Upregulation of PD-L1 in the PD-L1-positive tumor cells may occur via adaptive immune resistance. It may also be assumed that PTEN-positive tumor cells evade antitumor immunity by upregulating the expression of PD-L1.

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