Immune landscape evaluation of NSCLC clinical samples

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Method Article

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Abstract

The associated publication reports proteogenomic analysis of non-small cell lung cancer, where we identified molecular subtypes with distinct immune evasion mechanisms and therapeutic targets, and validated our classification method in separate clinical cohorts. This protocol describes histological, tertiary lymphoid structure (TLS), and immunohistochemical evaluation of clinical samples. Specifically, immunohistochemistry was performed for PD-L1, CD3, and CD8 on tumor microarrays (TMAs) derived from formalin-fixed paraffin embedded (FFPE) samples.

Introduction

Reagents

Equipment

Procedure

Sample collection and histological classification

Formalin-fixed paraffin embedded (FFPE) samples collected for histology of the early-stage NSCLC cohort (Lehtiö et al. associated publication) were evaluated with hematoxylin and eosin staining by a certified pathologist with extensive experience in lung pathology (HB). The classification was performed according to the World Health Organization Classification for Lung cancer, employing both 2004¹ and 2015² editions. Moreover, Tumor microarrays (TMA) were constructed from 1.0 mm punches of the FFPE lung cancer blocks described above, using a manual arrayer (Pathology Devices, Inc., Westminster, MD).

Immunohistochemistry for PD-L1, CD3 and CD8

Immunohistochemistry (IHC) for PD-L1 was performed on TMAs with the help of a Ventana Benchmark Ultra (Roche Diagnostics, Switzerland), pre-treating the tissue with Cell Conditioning 1 (cat. No. 950-124, Roche Diagnostics, Switzerland), incubating the section with the anti-PD-L1 antibody (rabbit monoclonal antibody clone 28-8, dilution 1:100, ab205921, Abcam, UK) and employing an OptiView DAB IHC Detection kit (cat. No. 760-700, Roche Diagnostics, Switzerland). IHC for CD3 and CD8 were always done on TMAs but instead of employing a DAKO immunostainer, the tissue was pre-treated with high pH Envision FLEX Target retrieval solution High pH (cat. No. K800421-2, DAKO, Denmark) in a PT-Link Module (DAKO, Denmark). Antibodies employed for the reactions were anti-CD3 (polyclonal rabbit antibody, cat. No. A0452, DAKO, Denmark) and anti-CD8 (mouse monoclonal antibody clone C8/144B, cat. No. M7103, DAKO, Denmark).

PD-L1 was evaluated according to the interpretation guidelines developed for the PD-L1 immunohistochemical test³ and were evaluated on 53 cases available on the TMAs. Briefly, a minimum

of 100 tumor cells were evaluated for each tumor sample (majority between 200 and 400), measuring the percentage of neoplastic cells that showed at least a partial and weak cell membrane positivity (Tumor Proportion Score, TPS). Any cytoplasmic staining was not evaluated; necrotic cells, immune cells and macrophages were not considered in the count. The presence of internal positive control was assessed on each sample, to assure the reliability of the immunohistochemical reaction.

CD3 and CD8 was evaluated in 90 cases available on the TMAs for immunohistochemical staining and evaluation. The manual annotation of these immunohistochemical markers was performed accordingly to Al-Shibli et al.⁴, considering the epithelial and the stromal compartments separated in the evaluation. Briefly, at least 100 nucleated cells were considered for each compartment of the sample and the percentage of positive cells in the membrane was counted. Samples with a percentage of positive cells inferior to 1 were considered negative.

Histology subtype and tertiary lymphoid tissue (TLS) evaluation on cluster 2 and 3

In order to explore the relationship between PD-L1 protein expression, the histological component and presence of TLSs, 21 cases were selected showing different expression of PD-L1 in the proteomic quantification (Lehtiö et al. associated publication). The histological classification was performed on hematoxylin and eosin sections, following the 2015 WHO classification of tumors of the lung². Focusing on the adenocarcinoma subtyping, the subtype percentages were registered by increments of 5%, according to Travis et al.⁵. A percentage was calculated for each of the 6 major adenocarcinoma subtypes (lepidic, acinar, papillary, micropapillary, solid, and invasive mucinous) in each tumor. For squamous carcinomas no further subtyping was performed. The tumor's bulk composition was manually annotated, dividing each tumor into epithelial, stromal, and immune compartments, and a percentage of necrosis was calculated. For intra-tumoral TLSs, 30 high-power fields were considered for counting the number of TLSs.

Troubleshooting

Time Taken

Anticipated Results

References

1. Travis, W. D., Brambilla, E., Muller-Hermelink, H. K. & Harris, C. C. *Pathology and Genetics: Tumours of the Lung, Pleura, Thymus and Heart*. (World Health Organization, 2004).
2. *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart*. Fourth edn, Vol. 7 (WHO Press, 2015).

3. Dako, A. (ed Agilent Technologies) (Agilent, United States, 2018).
4. Al-Shibli, K. I. *et al.* Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **14**, 5220-5227, doi:10.1158/1078-0432.ccr-08-0133 (2008).
5. Travis, W. D. *et al.* International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* **6**, 244-285, doi:10.1097/JTO.0b013e318206a221 (2011).