Extracellular matrix gene expression and cytotoxic T lymphocyte infiltration in the tumor microenvironment in non-small cell lung cancer

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Introduction

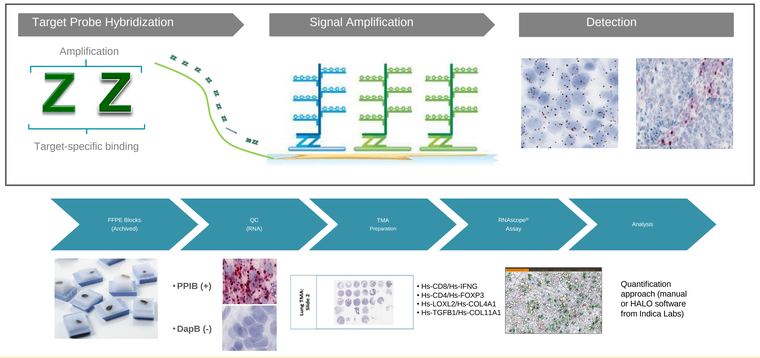
Immunotherapy has proven to be a powerful anti-tumor therapy, harnessing the body's own immune system to target and kill tumor cells. However, immunotherapy is not successful in all cancer patients due to both intrinsic non-responsiveness and adaptive resistance. Developing predictive biomarkers and understanding mechanisms of resistance are major goals of the immuno-oncology community. The extracellular matrix (ECM), an important factor for promoting tumor growth, survival, and migration of tumor cells, can also act as a physical barrier to prevent immune cell infiltration and promote tumor immune escape. Components of the ECM such as COL11A1, COL4A1, and LOXL2 have been shown to be associated with cancer progression. Furthermore, new data suggests that TGFβ activation leads to up-regulation of ECM genes in cancer-associated fibroblasts and immune suppression. However, it remains poorly understood which cells in the tumor microenvironment (TME) are the sources of ECM gene expression and how they are related to tumor infiltrating cytotoxic T lymphocytes (CTLs).

Design

RNAscope® ISH assay: we employed a highly sensitive and specific RNAscope *in situ* hybridization (ISH) duplex assay to directly visualize the tissue distribution of cells expressing *COL4A1*, *COL11A1, LOXL2, and TGFB1* in relation to tumor infiltrating CTLs in non-small cell lung carcinoma (NSCLC). NSCLC tissue microarrays (TMAs) consisting of 63 independent patient FFPE tumor samples were analyzed using this ISH assay with the following probe combinations: Hs-CD8/Hs-IFNG, Hs-CD4/Hs-FOXP3, Hs-LOXL2/Hs-COL4A1, and Hs-TGFB1/Hs-COL11A1.

Imaging and scores: Images were acquired using a Leica Biosystems Aperio AT2 Digital Pathology Slide Scanner. Semi-quantitative scoring was assigned according to ACD manual's recommendation (0, no staining or <1 dot for every 10 cells; 1, 1-3 dots/cell; 2, 4-10 dots/cell, very few dot clusters; 3, >10 dots/cell, less than 10% positive cells have dot clusters; 4, >10 dots/cell, more than 10% positive cells have dot clusters).

Figure 1. The RNAscope® assay and study workflow



Results

We observed *COL4A1* expression in both tumor and tumor-associated stromal cells in different samples. In contrast, *COL11A1* was only expressed in tumor-associated stromal cells. Interestingly, high *COL4A1* expression in tumor areas was associated with high CD8+ T cell infiltration, whereas high *COL11A1* expression was associated with poor CD8+ T cell infiltration. In addition, tumor expression of *TGFB1* was positively correlated with *COL11A1* expression.

Figure 2. Representative images of the correlation among COL4A1/LOXL2, COL11A1/TGFB and tumor infiltrated immune cells

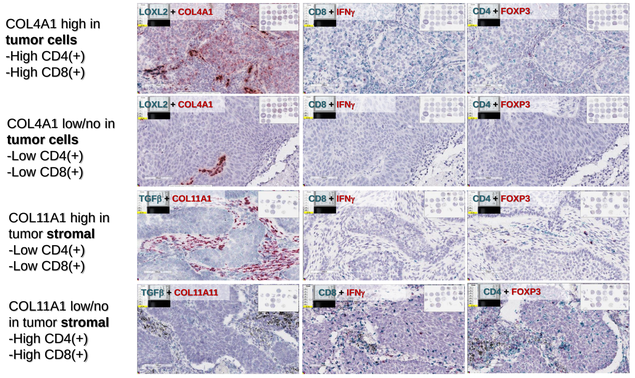
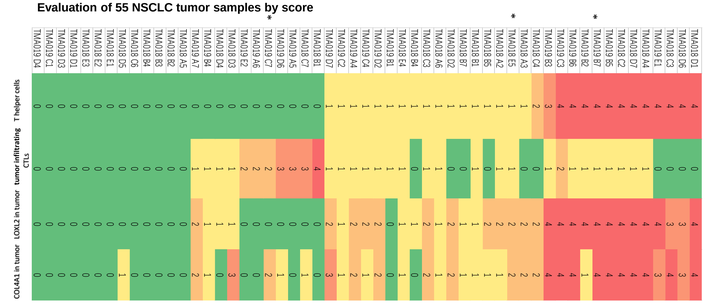


Figure 3. The distribution characteristics of COL4A1, LOXL2 and tumor infiltrating cytotoxic T lymphocytes and T helper cells



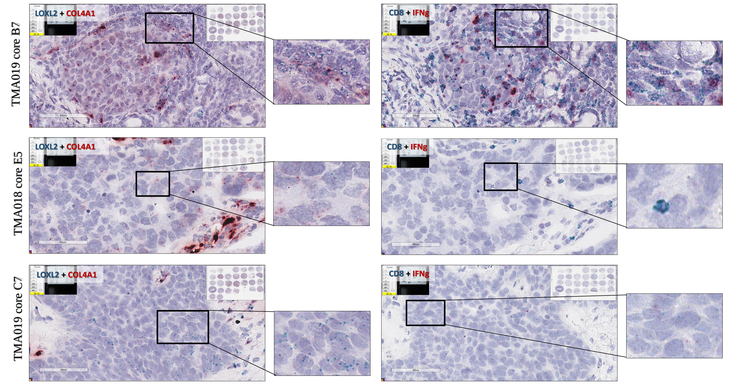
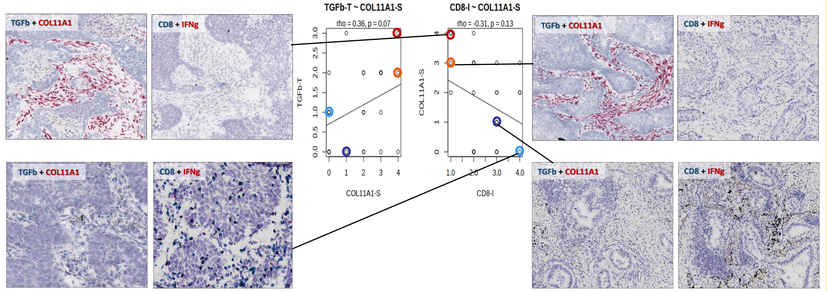


Figure 4. The distribution characteristics of COL11A1, TGFβ and tumor infiltrating cytotoxic T lymphocytes



Conclusion

These data depict a complex landscape of ECM gene expression and their relationship to T cell infiltration in the tumor and TME.

- A trend of positive correlation between tumor cell expression of COL4A1 and CD8(+) immune cell infiltration, and a trend of negative correlation between stromal COL11A1 and CD8(+) immune cell infiltration was observed in NSCLC tissues;
- When no COL4A1 was expressed in tumor cells, high tumor cell expression of LOXL2 was correlated with low CD8 (+) immune cell infiltration in NSCLC tissues;
- Tumor cell expression of TGFβ was correlated with high COL11A1 in stroma in NSCLC tissues.

Taken together, these results demonstrate that the RNAscope ® assay provides a powerful approach to directly examine the interactions between tumor, ECM, and T cell immune infiltration, and offers advantages over immunohistochemistry (IHC) for identifying the cellular sources of secreted proteins such as ECM components in the TME.