Optimization and validation of immunohistochemical assays for the detection of TREM2 and evaluation by image analysis in FFPE human tissue


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Abstract

Introduction

The tumor microenvironment (TME) often contains high levels of suppressive myeloid cells that may adversely affect the efficacy and overall survival of patients. Tumor microenvironment reprogramming (TMR) approaches involve altering the composition and function of myeloid cells in the TME. Pionyr has identified the immunoreceptor tyrosine-based inhibitory motif (ITIM) domain-containing protein 1 (CD330/TREM2) as a highly enriched target on tumor-associated macrophages (TAMs) and has developed an anti-TREM2 therapeutic monoclonal antibody. Anti-TREM2 Abs were shown to achieve partial tumor regression in vivo and to improve overall survival in a preclinical model. In this study, we aimed to develop a multiplexed immunohistochemistry (IHC) assay using Pionyr’s CD330/TREM2 antibody to validate the detection of TREM2 in human breast and colon colorectal (CRC) tumor samples. Moreover, to further understand the role of TREM2 in the tumor and stromal interaction with other immune cells, multiple immunohistochemical (IHC) panels were developed.

Methods

The multiplexed TREM2 IHC assay was optimized and validated at NovoRex Laboratories in a CALU-1 conditioned and GAP-activated labeling using an anti-TREM2 antibody from a commercial available source, as well as a secondary antibody from Abcam. Optimization on controls and patient samples was carried out using immunohistochemistry experiments. The assay was validated for sensitivity, specificity, and intraday precision. Tumor samples were evaluated both manually and using a computer-aided image analysis system. Tissue sections were cut using a microtome, and images were further processed digitally and analyzed using internal software.

Results

The multiplexed TREM2 IHC assay was successfully optimized and validated at NovoRex Laboratories and showed excellent specificity, sensitivity, and precision following pathologist-guided image analysis. The optimal anti-TREM2 staining concentration was 1 μg/mL, with a high signal-to-noise ratio. An additional panel of human tumor samples, the average TREM2 expression was highest in renal cell carcinoma, followed by ovarian, breast, lung, gastric, and colorectal adenocarcinoma. TREM2 expression was absent from most tumor tissues. The intensity of staining was variable between different human tumor tissues. Staining was performed using an anti-TREM2 monoclonal antibody, and uninvolved immune cells were used as an internal control for determining the percentage TREM2+ cells in the total tumor area. The anti-TREM2 antibody was successfully visualized and quantified using the ImageJ multiplex panel.

Conclusions

Overall, the TREM2 IHC assay demonstrated that TREM2 IHC were highly enriched in the TME of the pruned breast and colon tumor biopsies undergoing biopsies. The multiplexed TREM2 IHC assay was successfully used on FFPE tissue samples from breast and colon tumors. Furthermore, the multiplexed TREM2 IHC assay was highly enriched in the stromal and tumor tissues.

Using image analysis to understand the spatial localization of TREM2 and in relation to other markers in the tumor microenvironment

We used our multiplex IHC assay to assess the coexpression of different cell types, subtypes, including TREM2, in the tumor and stromal compartments. We observed that CD330/TREM2 was highly expressed in the stromal and tumor tissues. The result suggests that CD330/TREM2 is strongly expressed in the stromal and tumor tissues.

Summary & Acknowledgements

The TREM2 expression was evaluated using the MyH2O IHC assay on FFPE tissue samples from breast and colon tumor biopsies. The multiplexed TREM2 IHC assay was successfully used on FFPE tissue samples from breast and colon tumors. The result suggests that CD330/TREM2 is strongly expressed in the stromal and tumor tissues.

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