1931P Assessment of PD-1/PD-L1 colocalization in hepatocellular carcinoma (HCC) using bright-field double labeling and quantitative digital image analysis

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Background: Tumors may suppress host defenses by activating immune checkpoints (eg, the programed cell death [PD-1/PD-L1] pathway). Colocalization (CL) is a requirement for PD-1/PD-L1 interaction. PD-1/PD-L1 CL in tissue sections, as determined by immunohistochemistry (IHC), may be an indicator of PD-L1/PD-1 pathway activity.

Methods: We assessed CL of PD-L1 and PD-1 in situ by applying a novel duplex bright-field IHC technique on 49 formalin-fixed, paraffin-embedded HCC samples using digital image analysis (DIA; Definiens Tissue Studio®) to determine the percentage of single PD-1^+ and PD-$L1^+$ cells, PD-L1/PD-1 double-labeled cells, and PD-1^+ cells adjacent to $\geq$ 1 PD-$L1^+$ cells.

Results: All cases showed typical HCC morphology (low- to high-grade trabecular [4/49], pseudoglandular [1/49], solid [40/49], clear cell [2/49], or desmoplastic [2/49]). PD-L1 was largely observed in immune cell infiltrates. On average, 2.6% ± 3.6% (median, 1.5%) of the cells (immune + tumor) within the tumor area were PD-1^+, and 4.3% ± 5.5% (median, 1.9%) were PD-$L1^+$. There was considerable variation among samples in the number of PD-1^+ (range, 0.05%-21.2%) and PD-$L1^+$ (range, 0.2%-30.3%) cells. In 18/49 cases (37%), the number of PD-1^+ cells exceeded the number of PD-$L1^+$ cells; in 31/49 cases (63%), the number of PD-L1+ cells exceeded the number of PD-1^+ cells. PD-1/PD-L1 double-stained cells were present in 31/49 cases (63%), and 1.6% ± 4.1% (median, 0.13%) of the cells were double labeled, with considerable intersample variation (range, 0.5%-22.9%). Finally, 10.5% ± 8.03% (median, 9.4%) of PD-1^+ cells were in the immediate vicinity of a PD-$L1^+$ cell (range, 1.1%-43.3%).

Conclusions: By combining a novel duplex bright-field IHC technique with DIA, we quantitated the number/distribution of PD-1^+ and PD-$L1^+$ cells in HCC. Variation in the numbers of PD-1^+ and PD-$L1^+$ cells, and PD-1^+ cells with $\geq$1 PD-$L1^+$ adjacent cells, in HCC was seen. Future studies can use these techniques to explore the predictive potential of PD-L1/PD-1 expression in patients who are being considered for immunotherapy. Our proof of concept results suggest that the methods may also be applied for other tumors.

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