IHC Performance of Two Rabbit Anti-Human PD-L1 Monoclonal Antibodies

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Abstract

Programmed cell death 1 ligand 1 (PD-L1) is a type 1 transmembrane protein involved in the regulation of cellular and humoral immune responses. PD-L1 is mainly expressed in antigen presenting cells, placenta, and some tumors such as melanoma, diffuse large B-cell lymphoma, and carcinoma of the lung, colon, rectum, kidney, as well as other organs. In this study, we evaluated the performance of the two rabbit monoclonal anti-human PD-L1 antibodies, clone SP142 (Spring Bioscience) and clone E1L3N (Cell Signaling Technology) for immunohistochemistry (IHC) application in normal and tumor tissues. The antibody concentration for optimal detection of PD-L1 was much lower for clone SP142 (0.44 µg/ml) than clone E1L3N (28 µg/ml). PD-L1 protein expression from 119 cases of non-small cell lung carcinoma (NSCLC) was determined by IHC using these two clones. PD-L1 expression was detected in 49% (58/119) of cases for clone SP142, but only 42% (50/119) for clone E1L3N. The mean H score of the PD-L1 staining intensity from these 119 NSCLC cases was significantly higher in the tissues stained with clone SP142 than those stained with clone E1L3N. Some background and cross-reactivity in stomach, kidney, and nerve was seen in tissues stained with clone E1L3N, but not in tissues stained with SP142. The data from this study demonstrate that SP142 is more sensitive and specific than clone E1L3N.

Materials and Methods

Tissues - Formalin-fixed paraffin-embedded (FFPE) normal tissue array, tumor array, and 119 NSCLC cases (previously undetermined for PD-L1 expression).

Antibodies - Rabbit anti-PD-L1 monoclonal antibodies: clone SP142 from Spring Bioscience, Pleasanton, CA and clone E1L3N from Cell Signaling Technology, Danvers, MA.

Western Blot - Karpas, H820 and Calu-3 cell lysate were used.

IHC protocol

Deparaffinization - Deparaffinize slides using xylene alternative and graded alcohols.

Antibody Dilution - Serial dilution (0.11 to 28 ug/ml) was made (images are shown in Figure 2) and 0.44 ug/ml was chosen for optimal staining for clone SP 142 and 28 ug/ml for clone E1L3N (images are shown in Figure 3-5).

Antigen Retrieval - Boil tissue section in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min.

Primary Antibody Incubation - Incubate for 10 minutes at room temperature.

Detection – 15 minutes using Reveal Goat Anti-Rabbit Detection Kit from Spring Bioscience.

DAB – 10 minute incubation.

Scoring Method. PD-L1 positive case = case with 5% or more of tumor cells with PD-L1 membrane staining.

H Score for PD-L1 IHC =3 x percentage of strongly staining membrane + 2 x percentage of moderately staining membrane + percentage of weakly staining membrane.

Platform - Lab Vision Autostainer.

Results –Western Blot for Clone SP142

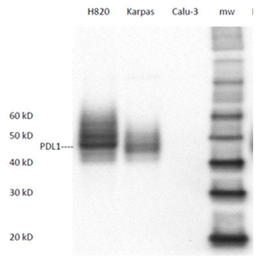

Figure 1. PD-L1 western blot using clone SP142. PD-L1 protein runs a smear around 45-55 kD in positive control H820 and Karpas cell lysate (possible due to post-translational modifications) and undetectable in negative control Calu-3 cell lysate.

Results – IHC Optimization in FFPE human tissues using clones SP142 and E1L3N

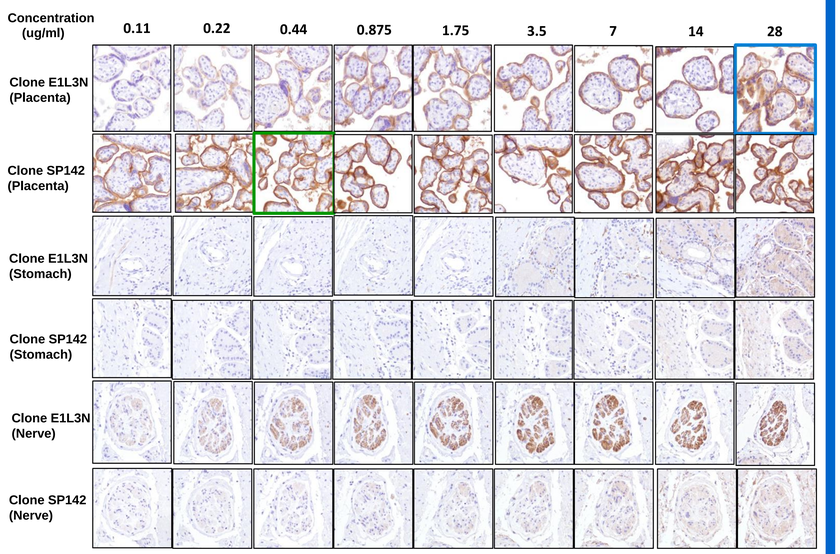

Figure 2. IHC protocol optimization in human tissues. A serial dilution (0.11-28 µg/ml) was made for both clones SP142 and E1L3N antibodies. To obtain an optimal staining in placenta, a much higher antibody concentration was required for clone E1L3N (28 µg/ml) than for SP142 (0.44 µg/ml). At 28 µg/ml, some cross-reactivity in stomach epithelial cells and nerve was observed in tissues stained with clone E1L3N, but not with clone SP142.

Results-Background in normal and tumor tissues

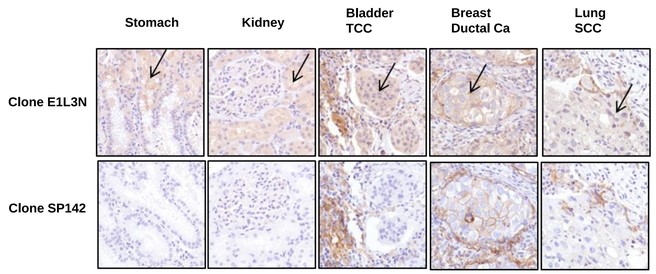

Figure 3. Illustration of background staining in normal and tumor tissues. Non-specific cytoplasmic staining (arrows) was noticed in stomach, kidney, bladder transitional cell carcinoma, breast ductal carcinoma, and lung squamous cell carcinoma stained by the CST clone E1L3N. In contrast, no background staining was observed in the tissues stained by SP142.

Results – PD-L1 Immunostaining in tonsil and cancer tissues

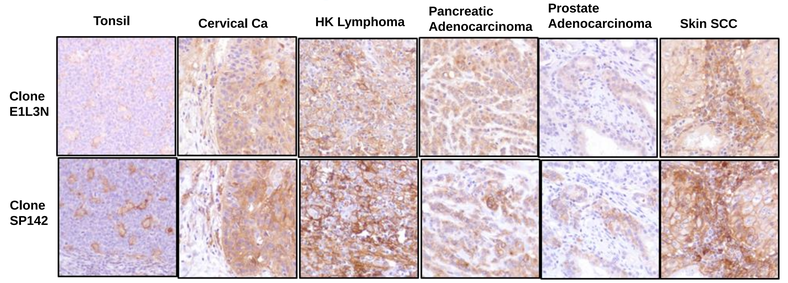

Figure 4. IHC staining in tonsil and various tumors. Specific membrane staining of macrophage, dendritic cells, and tumor cells was stronger in tissues stained with SP142 (lower panel) than E1L3N (upper panel).

Results – PD-L1 Immunostaining in lung squamous cell carcinoma

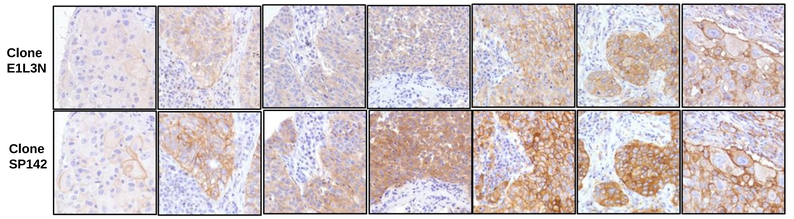

Figure 5. IHC staining of representative cases of lung squamous cell carcinoma. Sequential sections of 119 NSCLC cases were stained with both clone E1L3N and SP142. PD-L1 expression was detected in 49% (58/119) of cases for clone SP142, but only 42% (50/119) for clone E1L3N. Weak to moderate staining was observed in tissues stained with clone E1L3N (upper panel), while strong staining was observed in tissues stained with SP142 (lower panel). The mean H score from these 119 NSCLC cases was significantly lower for E1L3N (56±8) than the score for SP142 (100±11).

Conclusions

Rabbit anti-PD-L1 monoclonal antibody clone SP142 is highly sensitive and specific for detecting PD-L1 protein expression in FFPE tissues. These collective data demonstrate that SP142 is more sensitive and specific than E1L3N.

References

1. Taube JM, Klein AP, Brahmer JR, Xu H, Pan X, Kim JH, Chen L, Pardoll DM, Topalian SL, Anders RA. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res. 2014 Apr 8.
2. Velcheti V, Schalper KA, Carvajal DE, Anagnostou VK, Syrigos KN, Sznol M, Herbst RS, Gettinger SN, Chen L, Rimm DL. Programmed death ligand-1 expression in non-small cell lung cancer. Lab Invest. 2014 Jan;94(1):107-16.

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