Accelerated Instability Testing Reveals Quantitative Mass Spectrometry Overcomes Specimen Storage Limitations Associated with PD-L1 Immunohistochemistry

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Background

Immunohistochemistry (IHC) in formalin-fixed, paraffin embedded (FFPE) tissue is widely used in clinical and research settings, but has limitations relating to epitope masking, post-translational modification and immunoreactivity loss that occurs in stored tissue by poorly characterized mechanisms with various mechanisms suggested for this loss, including oxidation, humidity and high temperature.[1] PD-L1 IHC is particularly susceptible to epitope degradation and is an ideal model for understanding signal loss in stored FFPE.[2]

Method

We assessed 1,124 tissue sections to understand environmental factors contributing to immunoreactivity loss in stored tissues. PD-L1 IHC using 4 clones (22C3, 28-8, E1L3N, SP142) was assessed in stored FFPE of lung and gastric carcinomas. Accelerated aging of FFPE was achieved using increased humidity, oxygen and temperature. Quantitative mass spectrometry (MS) was used alongside IHC for quantifying PD-L1. Global proteome MS analyses were used to assess proteome-wide oxidation.

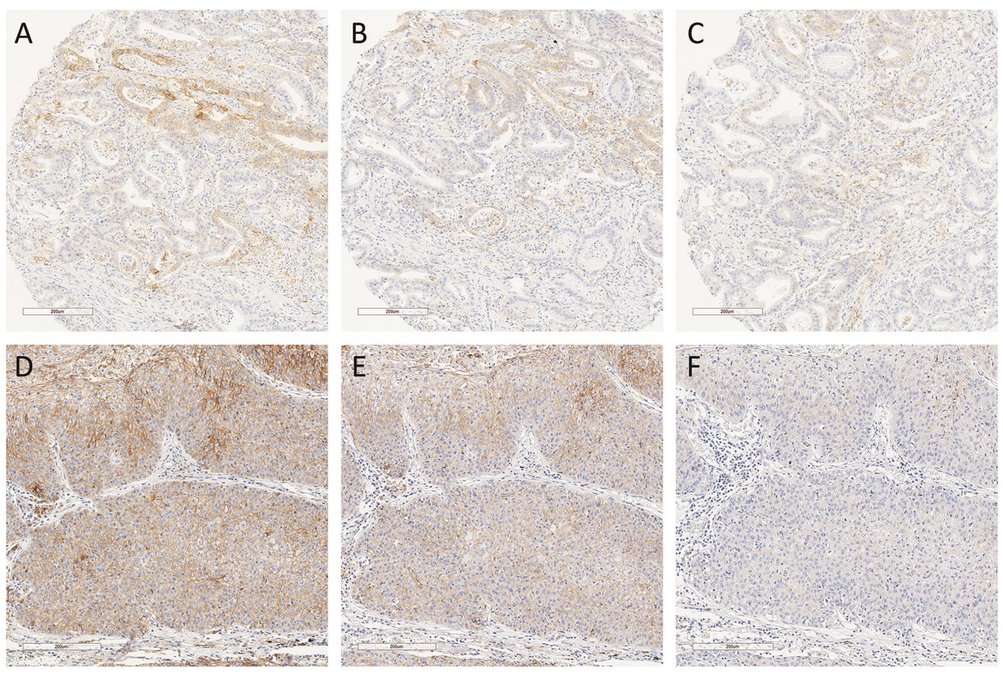

Fig 1. Representative PD-L1 expression assessed by E1L3N IHC in FFPE gastric carcinoma under normal atmospheric conditions (A-C) and in NSCLC under acceleration conditions (D-F). A – Day 0, B – 4.5 months, C – 24 months; D – Day 0, E – Day 9, F – Day 28.

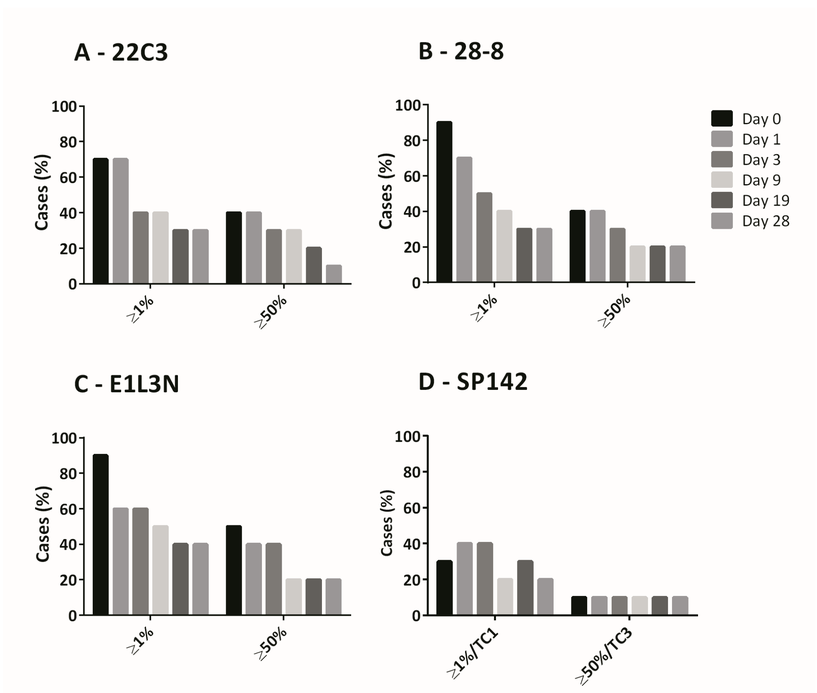

Fig 2. PD-L1 by clinical cut-offs in FFPE NSCLC sections over time in the acceleration chamber with conditions of 100% oxygen, 80% humidity, and 37°C for 22C3, 28-8, E1L3N and SP142 PD-L1 clones. Bars represent number of cases in series with PD-L1 expression equal or above TPS clinical cut-off thresholds.

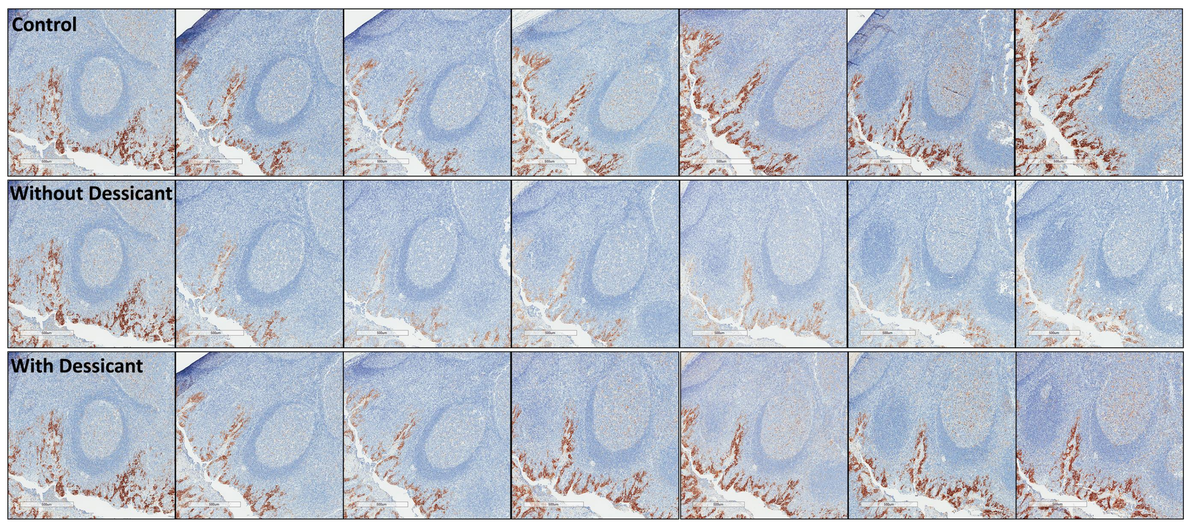

Fig 4. PD-L1 expression by E1L3N in FFPE tonsil sections at days 0, 1, 3, 7, 14, 21, and 28. The first row shows tissue sections stored under normal ambient conditions, the second and third row shows tissue sections within an incubator at 100% oxygen, 37°C and 80% humidity without (second row) and with (third row) desiccant.

Results

Increased humidity and temperature resulted in significant acceleration of immunoreactivity loss (Fig 1, Fig 3), which is largely mitigated by the use of desiccants (Fig 4). 22C3 and 28-8 were most susceptible to signal loss, with E1L3N the most robust (56%, 58% and 33% reduction $p<0.05$) (Fig 2). MS quantification of PD-L1 correlated strongly with IHC expression on unaged sections ($R2=0.745$ $P<0.001$) with MS demonstrating no loss of PD-L1 protein Fig 5), even in sections with significant staining loss by IHC. MS demonstrated a significant but only modest oxidation of proteins by global analyses, including PD-L1. (Fig 6)

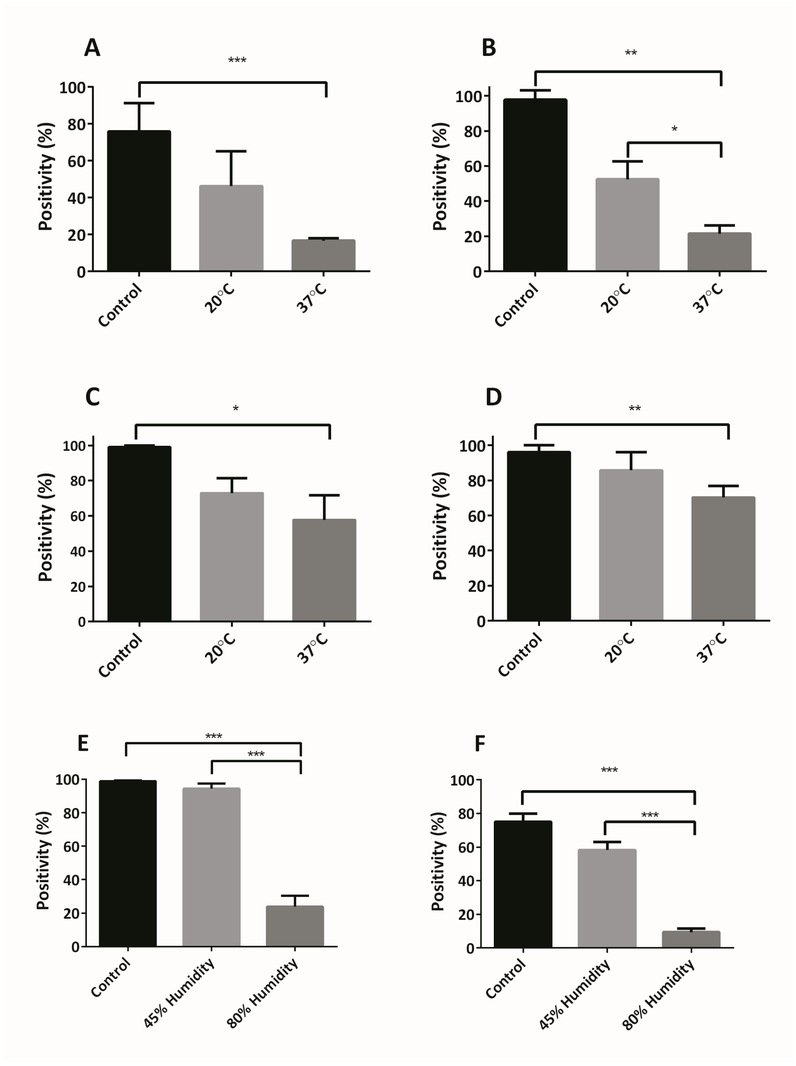

Fig 3. Placenta and tonsil FFPE sections incubated in the acceleration chamber under different environmental conditions at day 28; A-D: 100% oxygen and 80% humidity at either 20°C or 37°C, then stained for PD-L1 (E1L3N) or pan-CK (AE1/AE3): A – Placenta PD-L1, B – Tonsil PD-L1, C – Placenta pan-CK, D – Tonsil pan-CK. E-F: 100% oxygen and 37°C at either 45% or 80% humidity at day 28, E – Placenta PD-L1, F – Tonsil PD-L1. Control conditions: 20°C, atmospheric humidity and oxygen. Bar represents mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.

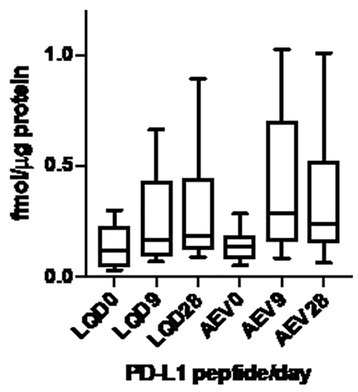

Fig 5. MS quantitation of PD-L1 peptides LQDAGVYR (LQD) and AEVIWTSSDHQVLSGK (AEV) in FFPE sections incubated in accelerated loss chamber at baseline (0), 9 and 28 days of incubation

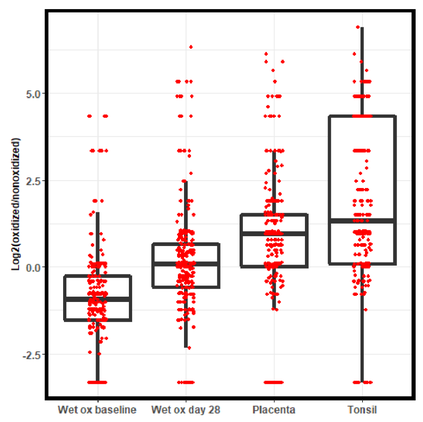

Fig 6. Global proteome analyses to assess proteome-wide oxidation under baseline (wet ox baseline) and acceleration conditions (wet ox day 28) compared to samples of naturally aged placenta and tonsil tissue stored under normal ambient conditions. The plotted values are log2 ratios of numbers of MS/MS spectra corresponding to oxidized and unoxidized methionine-containing peptides. Higher log2 ratios correspond to greater extent of proteome oxidation.

Conclusion

Immunoreactivity loss appears to be largely driven by the presence of humidity and temperature, with oxidation only playing a minor role. Structural distortion of epitopes maybe the mechanism of action for this, rendering them unsuitable for antibody binding following epitope retrieval. Limitations of IHC for biomarker analysis in stored tissue sections can be complemented through the use of MS. In some situations, MS may be preferred for retrospective analyses of archival FFPE collections.

References

1. Xie R, Chung JY, Ylaya K, Williams RL, Guerrero N, Nakatsuka N, et al. Factors influencing the degradation of archival formalin-fixed paraffin-embedded tissue sections. J Histochem Cytochem 2011;59:356-365.

2. Sato Y, Fujimoto D, Uehara K, Kawachi H, Nagata K, Nakagawa A, et al. Reduced Tumour Proportion Scores for Programmed Cell Death Ligand 1 in Stored Paraffin Tissue Sections. Anticancer Res 2018;38:1401-1405

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