Programmed Death 1 (PD1) and Programmed Death Ligand 1 (PDL1) are new targets of immunotherapy with PDL1 emerging as a potential predictive biomarker for response to PD1/PDL1-directed cancer therapies. PDL1 characterization represents a challenge due to variation based on tumor heterogeneity and dynamic changes in the microenvironment as well as technical limitations associated with its detection. To determine the reliability of detecting PD1/PDL1 in breast cancer using immunohistochemistry (IHC), we analyzed a series of paraffin-embedded tumor samples from 116 untreated patients diagnosed between 2001 and 2013 (44% luminal A, 28% luminal B, 15% triple-negative and 15% HER2). We employed a double-IHC stain using a polyclonal antibody (Ab) targeting PD1 and a monoclonal Ab targeting PDL1 (E1L3N clone). Additionally, to correlate PD1/PDL1 expression with the extent of tumor infiltrating lymphocytes (TIL) and their organization in tertiary lymphoid structures (TLS), we performed a second double-IHC stain with antibodies to CD3 and CD20, which are pan T and B cell markers, respectively. Membrane PDL1 positivity has been evaluated as the percentage of positive cells among TIL, neoplastic and stromal cells. PDL1+ was defined as >1% of positive cells and PD1 was considered positive when detected in >5% of TIL. The pathological assessment was performed by a well-trained pathologist blinded from the clinical data. We found that 22% of the cases were PDL1+ (41% triple-negative, 28% luminal B, 25% HER2 and 10% luminal A) and that this molecule was more frequently expressed on TIL (18%) then neoplastic (7%) or stromal cells (3%) (p = 0.0002). PDL1 expression was significantly and positively associated with PD1 (p = 0.0005), Ki67 (p < 0.0001), TIL infiltration (p = 0.01) and TLS presence (p= 0.006). Our study demonstrates that the level of tumor immune infiltration (evaluated for the extent of TIL and their organization in TLS) is correlated with the expression of potentially targetable immune check-point molecules such as PD1 and PDL1, signifying that they are not only prognostic immune-biomarkers but could also be helpful for identifying patients that could clinically benefit from immunotherapy.

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