Original Article
Programmed death ligand 1 (PDL-1) expression in triple negative breast cancer cells: new proposal for a "tumor score" definition

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Abstract: Recently, several studies showed that PDL-1 pathway may have a key role in the interaction of tumor cells with host immune response, and PDL-1 expression of tumor cells may serve as a mechanism of adaptive immune resistance. The data available in the literature about PDL-1 immunohistochemical expression in tumor tissues are not uniform, for the use of different antibodies clones and the absence of a standardized operative protocol. In this study we analyzed PDL-1 expression in a series of triple negative breast cancers and defined a standardized protocol suggesting a "tumor score" for its evaluation. Its application on large tumor case series will allow to investigate the real prognostic value of PD-L1.

Keywords: PD-L1, TNBC, tumor score

Introduction

Triple negative breast cancers (TNBCs), characterized by tumors that do not express estrogen receptor (ER), progesterone receptor (PR), and HER-2 genes, account for 10%-24% of invasive breast cancers, and they are typically high-grade tumors with different histological types. Usually, patients with TNBC tend to have a higher recurrence rate after diagnosis, a short disease-free interval, reduced overall survival, especially for the lack of targeted therapies [1]. This lack of targeted therapies has intensified the interest in this group of patients and the research of new molecular signatures tailored to this specific subtype [2].

PD-L1 is a 40 kDa transmembrane protein that is expressed on a wide variety of normal tissues including natural killer cells, macrophages, myeloid dendritic cells, B cells, epithelial cells, and vascular endothelial cells [3]. Recently, several studies showed that PD-1/PD-L1 pathway may have a key role in the interaction of tumor cells with host immune response, and tumor cells PD-L1 expression may serve as a mechanism of adaptive immune

resistance. Many human cancers have been shown to express PD-L1 and in most of cases, its expression was also correlated with a poor prognosis. This supports the hypothesis that these molecules can represent potential prognostic and predictive biomarkers in many human solid tumors [4]. However, recent reports revealed that the expression of the PD-L1 on tumor cells are not uniform, for the use of different antibodies clones, with variable specificity, often doubtful topographical localization and with a score not uniquely defined.

A case series of 20 TNBCs that underwent a mastectomy, quadrantectomy or metastectomy at the National Cancer Institute "Giovanni Pascale Foundation" of Naples, Italy, were enrolled into this study. In all samples we detected PDL-1 immunohistochemical expression (human PD-L1 rabbit monoclonal antibody [SP263] Ventana, Tucson, AZ), to define a correct operative protocol and especially an adequate score of interpretation, not being yet described in the literature.

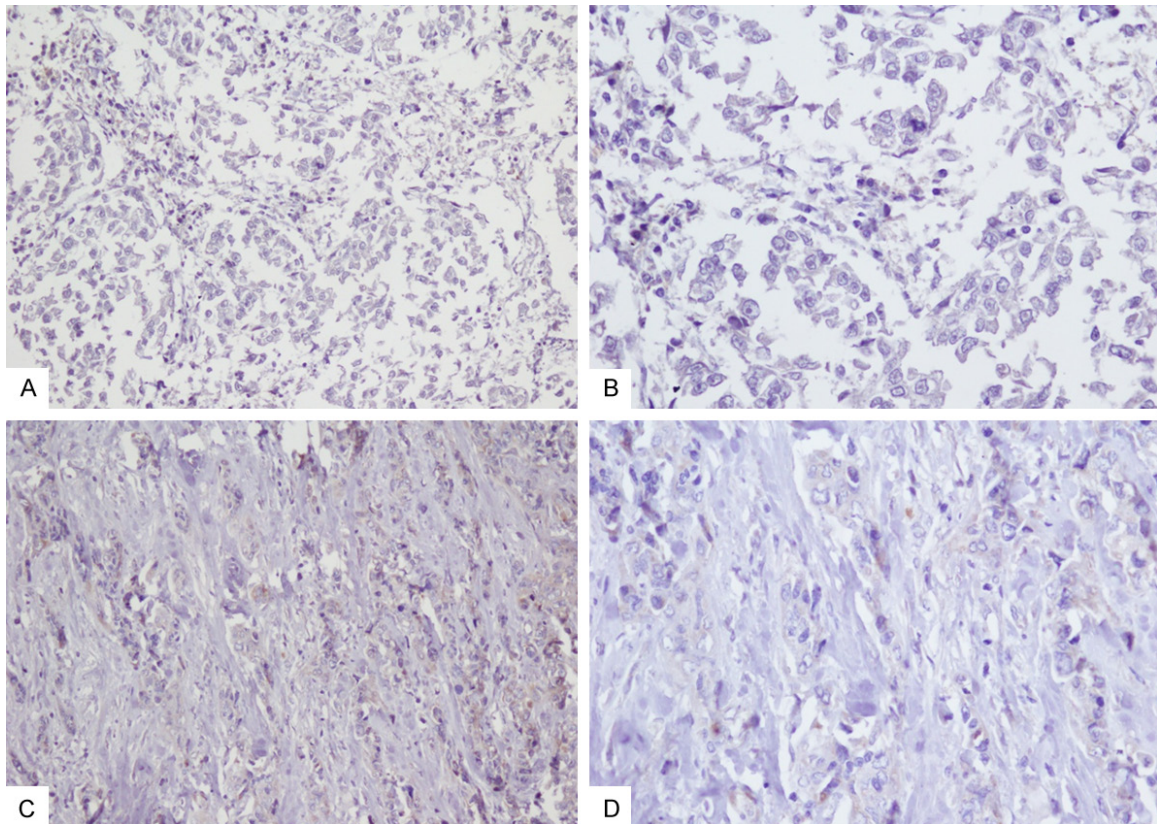Our results showed heterogeneous expression of PD-L1 on TNBC tumor cells. For its assess-

Figure 1. "Score 0" PD-L1 immunohistochemical staining: A: Absence of membranous immunoreactivity 20×; B: Detail of absence of membranous immunoreactivity 40×; C: Mild/moderate cytoplasmic positivity 20×; D: Detail of mild/moderate cytoplasmic positivity 40×.
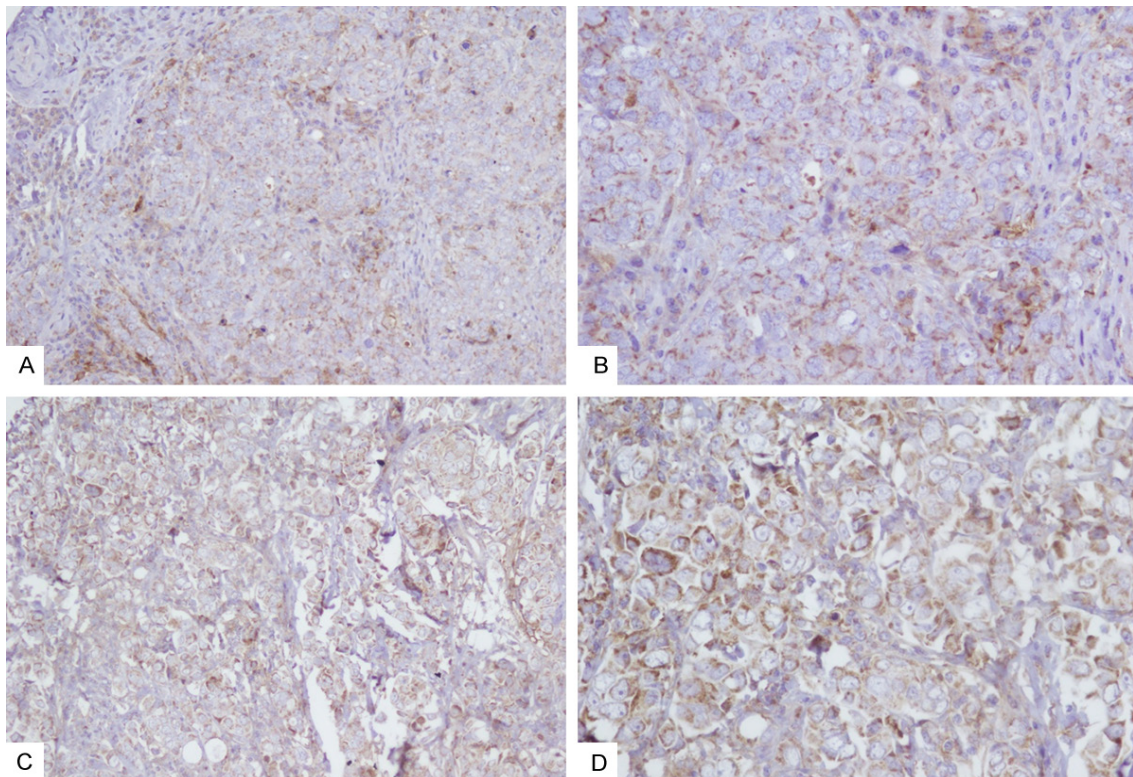
Figure 2. "Score 1" PD-L1 immunohistochemical staining: A: Incomplete membranous positivity, with a moderate/intense immunoreactivity, without cytoplasmic positivity 20×; B: Detail of incomplete membranous positivity with basolateral and/or with semicircular bars, without cytoplasmic positivity 40×; C: Incomplete membranous positivity, with a moderate/intense immunoreactivity, with cytoplasmic positivity 20×; D: Detail of incomplete membranous positivity with basolateral and/or with semicircular bars, with mild cytoplasmic positivity 40×.
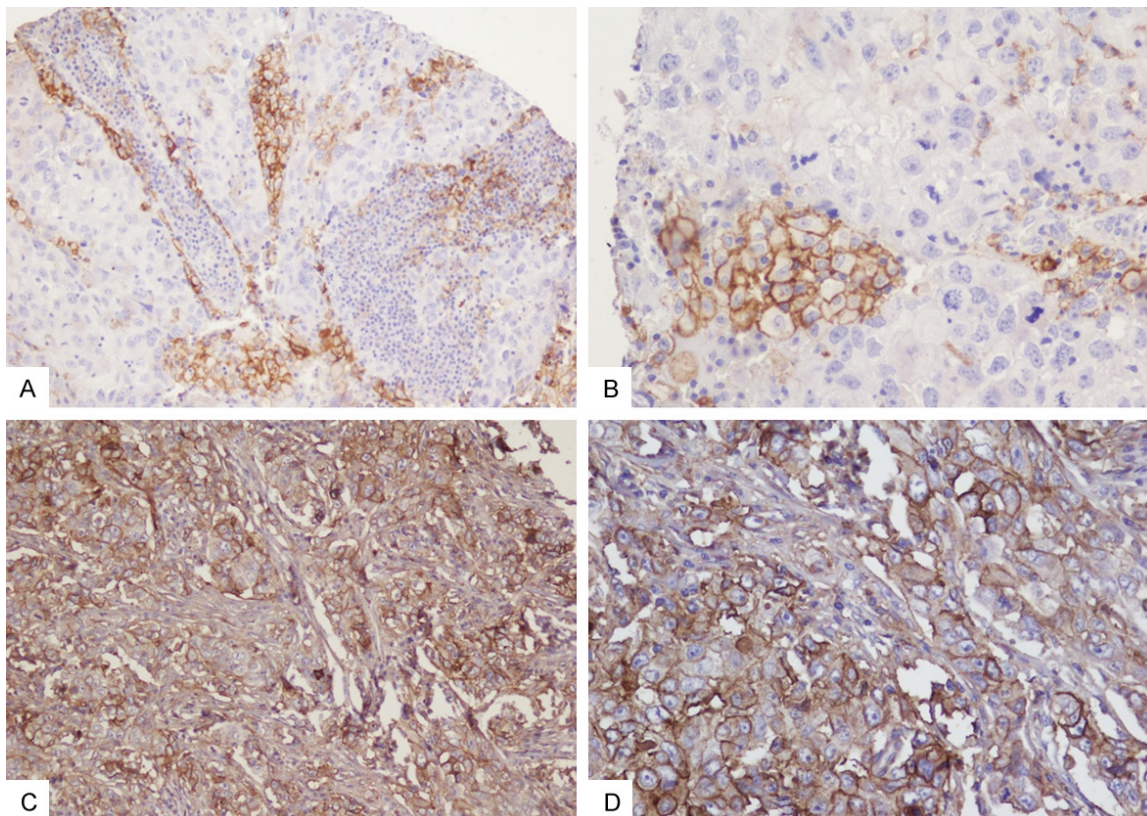

Figure 3. "Score 2" PD-L1 immunohistochemical staining: A: Complete membranous positivity, with a moderate/intense immunoreactivity, without cytoplasmic positivity 20×; B: Detail of complete membranous positivity, without cytoplasmic positivity 40×; C: Complete membranous positivity, with a moderate/intense immunoreactivity, with cytoplasmic positivity 20×; D: Detail of complete membranous positivity, with moderate/intense cytoplasmic positivity 40×.

ment and a score definition, we considered both a qualitative and a quantitative parameter. For the qualitative criteria we considered the immunoreactivity of membrane dividing it into "absent", "incomplete" and "complete", and the intensity of the reaction at the membrane level, dividing it into "mild", "moderate" and "intense". For the quantitative criteria we considered the percentage of positive tumor cells ≥ 10%.

The combination of the two parameters allowed us to establish as: "score 0" cases with the absence of membranous immunoreactivity (**Figure 1A**, **1B**) or mild/moderate cytoplasmic positivity (**Figure 1C**, **1D**); "Score 1+" cases with incomplete membranous positivity, which can be the basolateral and/or with semicircular bars, with a moderate/intense immunoreactivity, with/without cytoplasmic positivity, in ≥ 10% of tumor cells (**Figure 2**); "Score 2+" cases with complete membranous positivity, with a moderate/intense immunoreactivity, with/without cytoplasmic positivity, in ≥ 10% of tumor cells (**Figure 3**).

In literature only few papers described PD-L1 expression in BC subtypes. Soliman et al. analyzed PD-L1 expression in BC cell lines models showing its overexpression in particular in basal type BC [5]. Sabatier et al. analyzed PD-L1 expression in 45 breast cancer cell lines and in a large case series of 5454 BC using a DNA microarrays, demonstrating that its upreg-

ulation was associated with better survival and response to chemotherapy [6].

However, studies in which PD-L1 expression was examined by immunohistochemistry in BC are few. In a recent study, IHC PD-L1 expression in a case series of 650 BC samples was evaluated, highlighting that its expression was significantly associated with age, tumor size, lymph node status and worse OS. IHC results showed in all cases a strong cytoplasmic positivity that makes difficult and unclear its interpretation [7]. However in the only one study on TNBCs, the authors have also considered stromal and cytoplasmic positivity of PD-L1. Cytoplasmic positivity of PD-L1 was associated with a lower risk of breast cancer death, but the authors did not provide a clear explanation on the real value of this positivity in tumor cells [8].

In conclusion, only a correct standardization of operative protocols for the PDL1 determination in tumor cells in different cancer subtypes will allow to re-evaluate the prognostic value of this marker for determining whether it can be useful in therapeutic stratification of cancer patients addressed to immunotherapies.

Disclosure of conflict of interest

None.

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References

[1] Schmadeka R, Harmon BE, Singh M. Triple-negative breast carcinoma: current and emerging concepts. Am J Clin Pathol 2014; 141: 462-477.

[2] Elsamany S, Abdullah S. Triple-negative breast cancer: future prospects in diagnosis andmanagement. Med Oncol 2014; 31: 834-839.

[3] Sheppard KA, Fitz LJ, Lee JM, Benander C, George JA, Wooters J, Qiu Y, Jussif JM, Carter LL, Wood CR, Chaudhary D. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. FEBS Lett 2004; 574: 37-41.

[4] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012; 12: 252-64.

[5] Soliman H, Khalil F, Antonia S. PD-L1 expression is increased in a subset of basal type breast cancer cells. PLoS One 2014; 9: e88557.

[6] Sabatier R, Finetti P, Mamessier E, Adelaide J, Chaffanet M, Ali HR, Viens P, Caldas C, Birnbaum D, Bertucci F. Prognostic and predictive value of PDL1 expression in breast cancer. Oncotarget 2015; 6: 5449-64.

[7] Muenst S, Schaerli AR, Gao F, Däster S, Trella E, Droeser RA, Muraro MG, Zajac P, Zanetti R, Gillanders WE, Weber WP, Soysal SD. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat 2014; 146: 15-24.

[8] Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, Su X, Wang Y, Gonzalez-Angulo AM, Akcakanat A, Chawla A, Curran M, Hwu P, Sharma P, Litton JK, Molldrem JJ, Alatrash G. PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res 2014; 2: 361-70.