

Abstract

Production of Antibodies Binding Murine Nuclear PD-L1 [†]

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Abstract: PD1/PD-L1 antibody therapy is used in the clinic (atezolizumab, avelumab, durvalumab), but its effect is ambiguous, both in patients and in mouse models. The purpose of this work was to analyze the binding of commercial and laboratory-obtained monoclonal antibodies to PD-L1. Previously, we obtained monoclonal antibodies (clone B12) to the extracellular fragment of murine PD-L1 expressed in *E. Coli* (exPD-L1). The primary screening of monoclonal antibodies by flow cytometry showed a low B12 binding compared to commercial antibodies. Cytokine-stimulated 3D cultures, permeabilized cells, and Western blotting were used to elucidate the causes of low binding on living cells. The permeabilization of cells B16/F10 (mouse melanoma), EL-4 (mouse lymphoma), and COLO357 (human pancreatic cancer) showed the binding of both mouse and human B12 PD-L1 antibodies. With the help of Western blotting, these data were confirmed. However, commercial antibodies (BioLegend, clone 10F.9G2) in this method did not bind to the mouse recombinant exPD-L1 protein, but bound to cell lysates. Cell cultivation on an anti-adhesive polyHEMA substrate led to the binding of B12 antibodies to the surface of cells under 3D cultivation conditions. The stimulation of EL-4 and B16/F10 cells in 3D cultures and subsequent incubation with Cy3-labeled B12 antibodies led to the appearance of pronounced fluorescence in the perinuclear region of cells. Thus, the obtained monoclonal antibodies to mouse exPD-L1 are cross-reactive with human PD-L1 protein; PD-L1 protein translocates to the cell membrane when cultured under 3D conditions into the nucleus and, when stimulated by cytokines, its biosynthesis is activated. The difference in the binding of antibodies B12 and 10F.9G2 may indicate a difference in the conformation of PD-L1 translocated to the membrane. The traffic of intracellular PD-L1 into the nucleus upon activation may indicate the launch of de novo protein synthesis with an altered conformation.

Keywords: PD-L1; antibody; immune checkpoint inhibitors; immunology



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Reference

1. Goryunova, M.C.; Ryazantsev, D.Y.; Petrova, E.E.; Kostenko, V.V.; Makarova, A.O.; Kholodenko, R.V.; Ryabukhina, E.V.; Kalinovsky, D.V.; Kotsareva, O.D.; Svirshchevskaya, E.V. Expression of extracellular fragment of murine PD-L1 and production of antibodies to PD-L1. *Russ. J. Bioorganic Chem.* 2024, *in press*.

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