Production, purification and antitumor potency evaluation of ROR-1 x PD-L1 and Mesothelin x PD-L1 bispecific antibodies.

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Cancer is one of the leading causes of death in developed countries. For many years, one of the most promising forms of cancer treatment has been immunotherapy. Unfortunately, despite its highly dynamic development, there remains a significant group of cancers that are resistant to this type of therapy. ROR-1 and Mesothelin are proteins that have minimal presence in healthy adult tissues but are highly overexpressed in many different types of cancers, where they are involved in processes such as proliferation and survival. Moreover, their presence on the surface of cancer cells has been correlated with poorer patient prognoses in many cases. PD-L1, in turn, is one of the best-characterized immune checkpoint regulators, identified as a source of immune evasion for many types of cancer. The goal of this project was the production and analysis of the anti-cancer potential of a set of symmetric bispecific antibodies: ROR-1 x PD-L1 and Mesothelin x PD-L1 in IgG1 and IgG4 formats. The antibodies were produced using two mammalian expression systems: transient production in HEK293 cells and stable cell lines in CHO cells. The obtained bispecific antibodies exhibited the expected physicochemical properties and demonstrated the ability to bind molecular targets and block PD-1/PD-L1 interactions with similar efficacy to reference monospecific antibodies. Adding the studied antibodies to co-cultures of PBMCs and cancer cells (lines Panc1, Panc 05.04, BT20, and RL95-2) led to significantly increased cancer cell lysis compared to similar combinations of monospecific antibodies, with the level of lysis depending on the antibody concentration. Additionally, in co-cultures with Panc1 and RL95-2 cells, a significantly elevated level of cytokines (IL-10, TNFα, IFNγ, Granzyme B) and a reduction in regulatory T-cell subpopulations (CD4+ CD25+ CD127low FoxP3hi and CD4- FoxP3+) were observed. The Mesothelin x PD-L1 antibodies promoted a more effective anti-cancer response than the ROR-1 x PD-L1 antibodies, and IgG1 antibodies exhibited slightly higher activity than IgG4 antibodies. In summary, the use of bispecific antibodies for the simultaneous blockade of ROR-1 and PD-L1 or Mesothelin and PD-L1 receptors is an innovative and promising approach to cancer therapy. However, confirming their effectiveness in more complex models, particularly in vivo, remains essential.