Cold-inducible RNA binding protein as a vaccination platform against hepatocellular carcinoma

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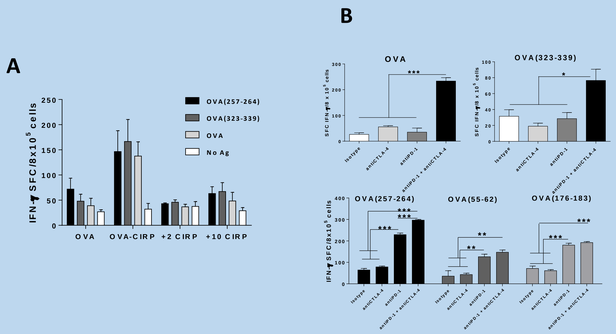
Introduction:

Lack of response to immune checkpoint inhibitors (ICPI) in cancer patients has been associated with poor lymphocytic infiltrate. Vaccines are known to promote tumor-specific immunity and are good candidates to enhance therapeutic responses to ICPI. Cold-inducible RNA binding protein (CIRP) is an endogenous TLR4 ligand that, upon linkage to antigens, increases their presentation to T lymphocytes, induces production of inflammatory cytokines and may render these antigens immunogenic. Blockade of PD-1/PD-L1 pathway, either alone or in combination with anti-CTLA-4 or anti-VEGF antibodies, has shown promising results in hepatocellular carcinoma (HCC) patients, although limited to a proportion of patients. Therefore, our aim was to generate a CIRP-based vaccine to induce responses against liver tumor antigens and thus increase and broaden the therapeutic efficacy of ICPI.

Methods:

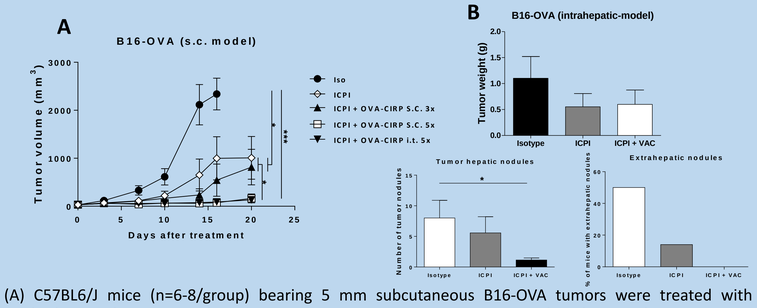
Immunogens containing antigens linked to CIRP were designed and expressed in bacteria and insect cells. They were used to vaccinate mice with or without ICPI (anti-PD-1 and/or anti-CTLA-4) and antigen-specific responses were determined by ELISPOT. Therapeutic efficacy of vaccines and combinations with ICI was tested in several murine subcutaneous and orthotopic liver cancer models.

Results I: Conjugation of Ag to the CIRP platform induces polyepitopic T cell responses which are enhanced by combination with ICPI



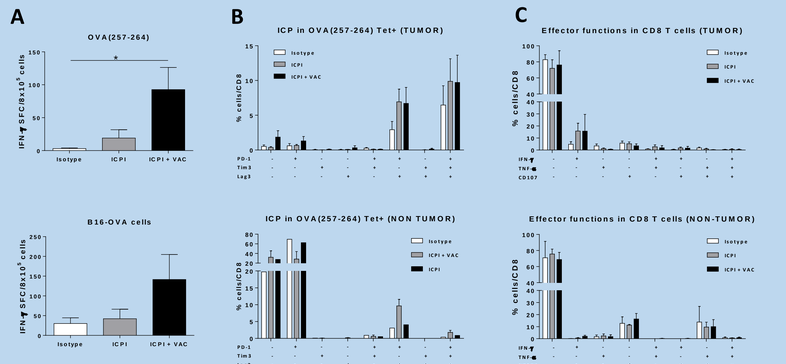
(A) C57BL6/J mice were immunized s.c. with 2 nanomoles of OVA, OVA conjugated to CIRP (OVA-CIRP), OVA plus CIRP (2 or 10 nanomoles each). One week later immune responses in the spleen were measured by IFN-gamma ELISPOT after stimulation with different OVA antigens. (B) OVA-CIRP was used as immunogen alone or in combination with ICPI antiCTLA-4, antiPD-1 or both antibodies. Responses against OVA protein, CD4 T cell epitope OVA(323-339), dominant CD8 T cell epitope 257-264 and subdominant CD8T cell epitopes 55-62 and 176-183 were measured as in A.

Results II: Immunization with OVA-CIRP enhances therapeutic responses induced by ICPI in subcutaneous and intrahepatic tumors



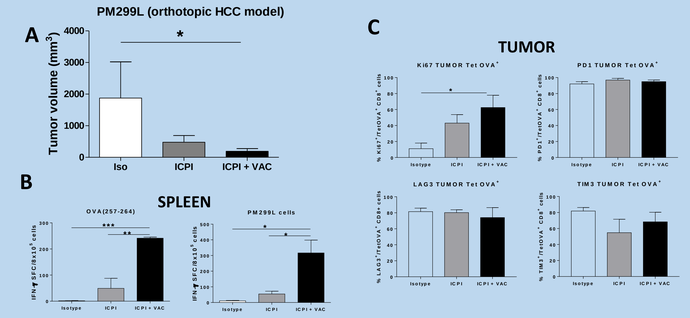
(A) C57BL6/J mice (n=6-8/group) bearing 5 mm subcutaneous B16-OVA tumors were treated with isotype antibodies at days 0, 7 and 14 (isotype, **UT**; antiCTLA-4 + antiPD-1, **ICPI**) with or without OVA-CIRP vaccine administered subcutaneously or intratumor, 3 or 5 times. Tumor volume was measured twice/week. (B) B16-OVA cells were injected in the liver of C57BL6/J mice (n=6) and one week later they received control or ICPI antibodies, or ICPI plus OVA-CIRP vaccine administered s.c. 5 times. Three weeks later livers were examined, analyzing the tumor weight, the number of tumor hepatic nodules as well as the percentage of mice without extrahepatic tumor nodules.

Results III: Combined treatment with OVA-CIRP and ICPI promotes antitumor T cell responses which are exhausted in the tumor



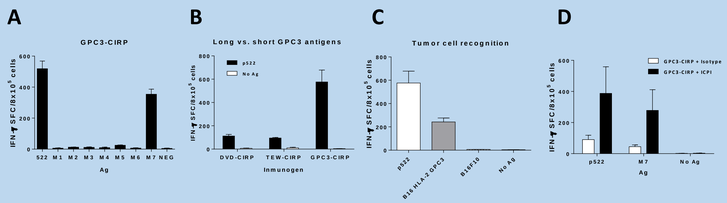
(A) Splenocytes from mice with hepatic tumors treated as in Results II were stimulated with CD8 T cell peptide OVA(257-264) or with irradiated B16-OVA tumor cells and responses were measured by ELISPOT. (B) CD8 T cells specific for OVA(257-264) in tumor (top panel) and non-tumor (bottom panel) liver tissue, labelled as Tet+, were analyzed by flow cytometry, determining the combined expression of exhaustion markers PD-1, Tim-3 and Lag3. (C) Intrahepatic lymphocytes from tumor (top panel) and non-tumor (bottom panel) tissue were stimulated with PMA and ionomycine and combined effector functions (expression of cytokines IFN-gamma and TNF-alpha, and of the cytotoxicity marker CD107) were determined by flow cytometry 4 h later.

Results IV: Immunization with OVA-CIRP enhances therapeutic responses induced by ICPI in an orthotopic HCC model associated with stronger antitumor immunity



(A) C57BL6/J mice (n=7-10/group) were injected in the liver with 5 x10^4 PM299L HCC cells. Four days later they received control or ICPI antibodies, or ICPI plus OVA-CIRP vaccine administered s.c. 5 times. Three weeks later livers were examined, analyzing the tumor volume. (B) Splenocytes from mice shown in A were stimulated with peptide OVA(257-264) or PM299L tumor cells and immune responses were analyzed by ELISPOT. (C) Proliferation (Ki67) and ICP (PD-1, LAG3 and TIM3) expression was measured in tumor-infiltrating TetOVA+ CD8 T cells in treated mice.

Results V: Immunization with human HCC antigen GPC3 conjugated to CIRP (GPC3-CIRP) induces anti GPC3 immunity, enhanced by combination with ICPI



(A) HHD-DR1 mice, transgenic for human HLA-A2 and HLA-DR*B1 molecules, were immunized with 2 nanomoles of HCC Ag GPC3 conjugated to CIRP (GPC3-CIRP) and one week later splenocytes were stimulated with 7 peptide pools encompassing the whole GPC3 sequence (M1 to M7) as well as with peptide GPC3(522-530) (contained in M7) and T cell responses were evaluated by ELISPOT. (B) Immunogenicity of entire GPC3-CIRP was compared with shorter Ag versions containing p522 plus different flanking regions conjugated to CIRP (DVD-CIRP and TEW-CIRP) in ELISPOT assays. (C) Recognition of tumor antigens was analyzed in ELISPOT assays by using parental B16F10 cells and B16F10 tumor cells transduced with human HLA-A2 molecules and with GPC3 Ag. (D) GPC3-CIRP vaccine was administered with control or IPCI antibodies and responses against p522 and M7 pool were evaluated.

Conclusion:

Vaccines based on liver tumor antigens conjugated to the CIRP platform may generate tumor-specific immune responses that enhance ICPI-mediated therapeutic effect, suggesting that they can be useful for combinatorial treatments in HCC. However, expression of ICP in tumor-infiltrating lymphocytes suggests that blockade of additional targets should be also considered.

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