AACR Annual meeting 2022

Development of an integrated *in vitro* and *in vivo* OVA-specific system for cancer vaccine discovery

No.5563

Jian Xiang, Jun Huang, Wenrong Zhou, Zhengang Peng, Qingyang Gu, Qunsheng Ji
Oncology & Immunology Unit, WuXi Biology, WuXi AppTec, China

Abstract

As the development of cancer immunotherapy and urgent demand of precision medicine, cancer vaccines have been envisioned as a novel and effective tool to cure cancer disease or prevent cancer from regression or metastasis. In order to support the research and development of new modality drug discovery, we have established an advanced and integrated *in vitro* and *in vivo* platform for various types of cancer vaccine, such as peptide vaccine, mRNA vaccine, DC vaccine and so on.

Ovalbumin (OVA) is a key model antigen which has been widely used in vaccination experiments. Here we established an OVA overexpressed system based on different types of tumor cells, such as B16F10-OVA and MC38-OVA. Firstly, OVA expression was confirmed by WB assay. Then, we proved that MHC-I:OVA complex were presented on the surface of stable cell lines successfully through IFN stimulation. The results of *in vitro* CD8$^+$ T cells activation and killing assay showed the T cells can be activated and target cells were specifically killed after the incubation, which indicates the presented OVA peptides can be utilized as an effective therapeutic target. OVA RNA vaccine was prepared by mixing OVA mRNA with special transfection reagents. Using ELISPOT assay, we demonstrated the immunogenicity of OVA-specific mRNA vaccine. Furthermore, *in vivo* tumor model of B16F10-OVA has also been established and the anti-tumor effect of vaccine was evaluated. The mechanism of tumor growth inhibition was also investigated via T cell, B cell and other cell populations depletion. Additionally, we explored the target protein biodistribution and *in vivo* behavior of mRNA vaccine carrier with Lumina imaging system.

Method

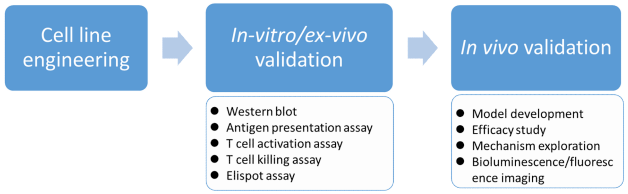

Fig 1. Scheme of OVA-specific system development and validation methods.

Results

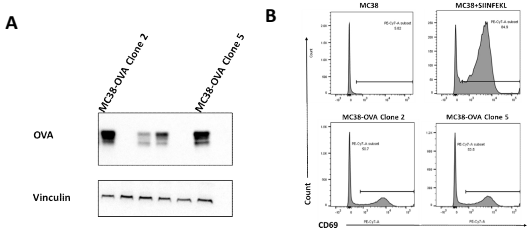

Fig 2. OVA expression & T cell activation assay on MC38-OVA clones

Subclones were picked up from MC38-OVA pool and Clone 2 and 5 showed strongest expression level (figure A). T cell activation status (CD69) was evaluated via co-culture of MC38-OVA cells and CD8$^+$ T cells isolated from OTI mice. From FACS data, significant peak shift of CD69 signals was observed in both clones, which indicated expressed OVA can be utilized as a target for treatment (figure B).

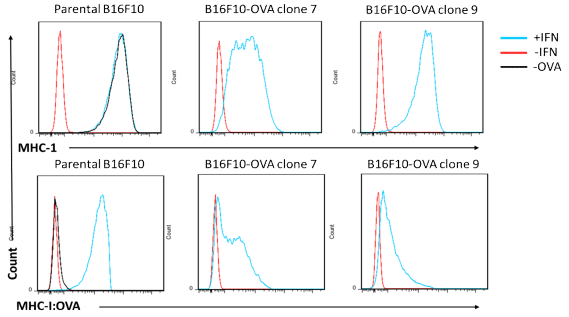

Fig 3. MHC-I:SIINFEKL complex presentation in B16F10-OVA clones

Subclones were picked up from B16F10-OVA pool and Clone 7 and 9 were used for presentation assay. After the IFN treatment, MHC-I and MHC-I:SIINFEKL complex were presented on both clones.

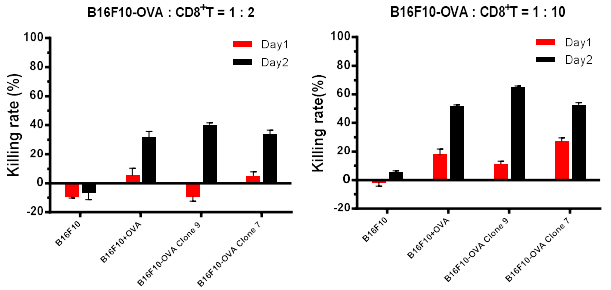

Fig 4. CD8$^+$T killing assay for B16F10-OVA clones

CD8$^+$ T cells isolated from OTI mice were co-cultured with B16F10-OVA clones at different E:T ratios for killing assay. After 1 or 2 days of incubation, B16F10-OVA cells were specially killed. Killing rate =(AVG. CTG_{Tumor}-Inividual $CTG_{Tumor+T}$)/AVG. CTG_{Tumor}

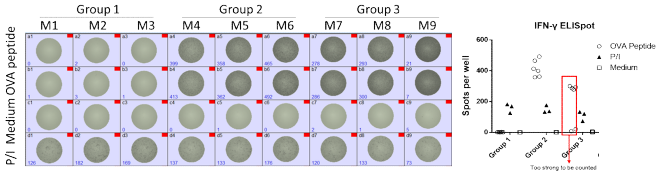

Fig 5. ELISPOT analysis post OVA RNA vaccine immunization

Animals were divided into 3 groups and each group was immunized with sterile water, 10 ug/mouse OVA mRNA and 20 ug/mouse OVA mRNA, respectively. OVA peptides, medium or P/I was used to re-stimulated the splenocytes. ELISPOT results showed that OVA-specific immune responses can be cascaded after OVA mRNA vaccines administration.

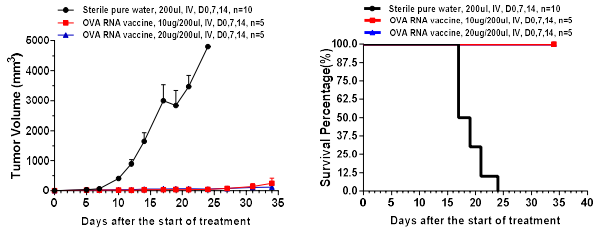

Fig 6. *In vivo* anti-tumor activity of OVA RNA vaccine in B16F10-OVA model

OVA RNA vaccine was *i.v.* injected into mice along with the B16F10-OVA cell inoculation. Treatment significantly inhibited tumor growth and prolonged the survival of tumor-bearing mice.

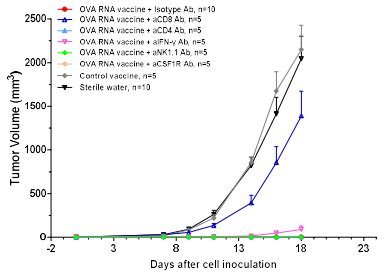

Fig 7. Cell populations depletion in efficacy study

Various of blocking antibodies were utilized for depletion of different cell populations in B16F10-OVA tumor-bearing mice. According to the results, CD8$^+$ T cells have significant impact on the anti-tumor activity of OVA RNA vaccine.

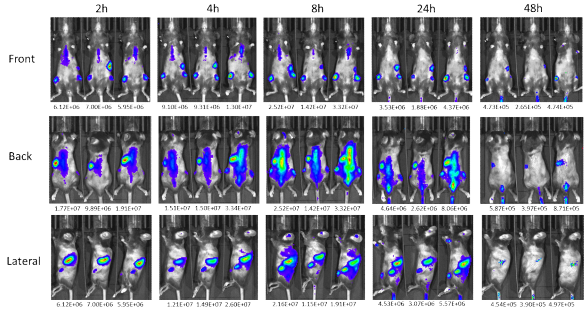

Fig 8. *In vivo* mRNA tracking via bioluminescence imaging

Luciferase RNA vaccine was *i.v.* injected into C57BL/6 mice. Bioluminescence images were captured at 2h, 4h, 8h, 24h and 48h post injection. From 3 dimensions of view, signals were mainly accumulated in spleen and bone marrow. The expression level of luciferase remained in a relative high level within the first 24h post injection.

Summary

In summary, the integrated OVA-specific system can be used for new adjuvants and peptide/mRNA carrier exploration in cancer vaccine discovery.