Engineered Oncolytic Viruses Expressing Immunomodulatory Genes Enhances In Vitro Anti-Tumor Cellular Immune Responses

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Abstract

While chemo- and radio-therapy are considered the first-line of treatment for most cancers, its serious side effects have led to continuous innovation and discovery of new anti-tumor treatments. Oncolytic viruses (OVs) possess a magnitude of mechanism of action to elicit anti-tumor immune response, including OVs innate cytotoxicity and enhanced anti-tumor immunity by inserting immunomodulatory genes. Moreover, there are no deaths or serious adverse events in clinical so far.

Here we evaluated the effect of modified OVs (mOVs) with various immunomodulatory genes on oncolytic capability and cellular immune responses *in vitro.* Our results indicate mOVs infected cancer cells more readily activates various immune cells, including T, natural killer (NK) and monocyte-derived dendritic cells (moDC), promoting a stronger response in immune cell proliferation and secretion of inflammatory cytokines such as IFN-γ.

Methods

Target validation, oncolytic capability and in vitro safety study

- Flow cytometry
- Enzyme-linked immunosorbent assay (ELISA)
- Cell Counting Kit-8 assay (CCK-8)
- Plaque assay
- Real-time PCR
- Apoptosis

In vitro immunomodulatory function study

- OV-treated moDC cells activate CD8⁺ T cells detection (ELISA, FACS)
- Detection of activation of T cell and NK cell by OV-infected cell supernatants.
- Detection of activation of PBMCs of different species by OV-infected cell supernatants.

Figure 1: Methods for in vitro evaluation of the efficacy of OV.

Results

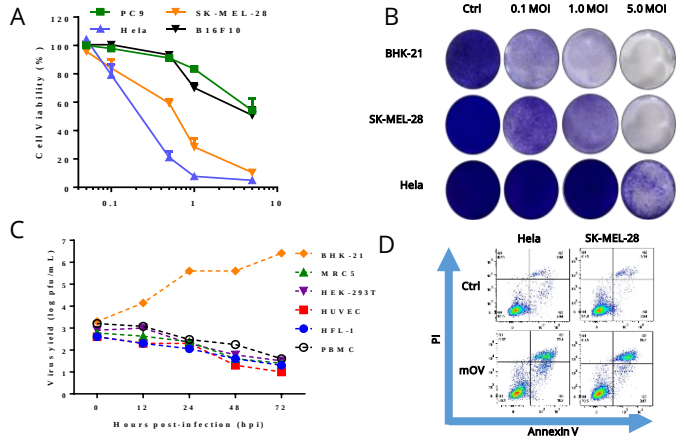

Figure 2: Detection of oncolysis, apoptosis, and in vitro safety analysis of mOV. Oncolytic efficacy of tumor cells infected with mOV were evaluated by (A) CCK-8 assay and (B) plaque assay, with (C) supernatant and cells collected at 0, 12, 24, 48 and 72 hpi used for virus detection by plaque assay. (D) Apoptosis of mOV infected cells were detected by FCM.

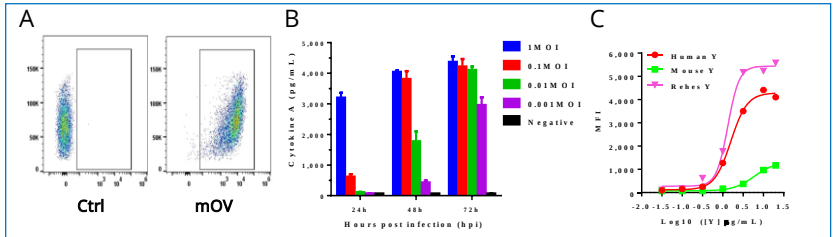

Figure 3: Target validation. (A) Expression of transgenes and cytokine secretion of mOV-infected tumor cells were evaluated by FCM and ELISA, respectively. (C) Affinity detection of Protein X expressed by mOV-infected tumor cells and its ligand Y of different species.

References

Hemminki O, Manuel dos Santos J, Hemminki A. Oncolytic viruses for cancer immunotherapy. J Hema Onco. 2020; 13:84

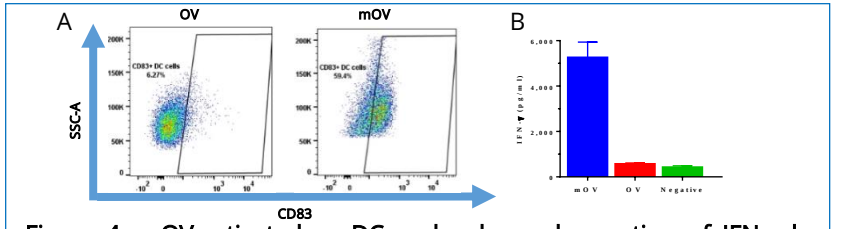

Figure 4: mOV-activated moDC and enhanced secretion of IFN-γ by CD8+ T cells. (A) Maturation of m/OV-treated DC by FCM. (B) m/OV treated DCs were co-cultured with CD8+ T cells for 72 h, IFN-γ was detected by ELISA assay.

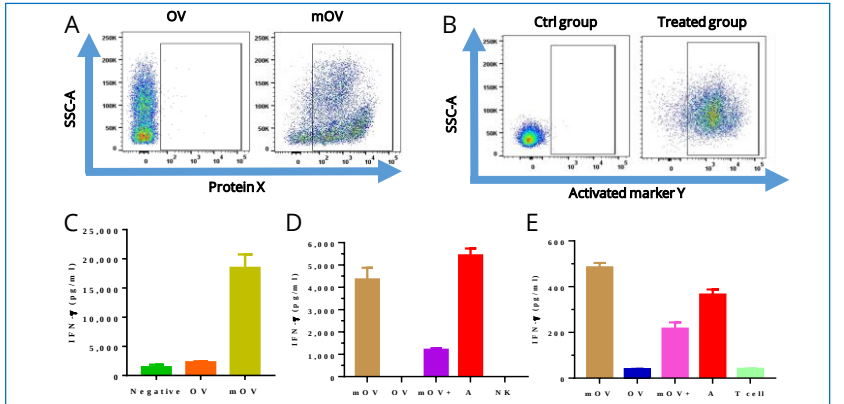

Figure 5: Immune activation of T and NK cells by m/OV virus. Expression of Protein X (A) and activated marker Y (B) with m/OV-infected tumor cells co-cultured with activated CD3+ T cells for 72 h, with (C) IFN-γ detected by ELISA assay. Supernatant of m/OV-infected tumor cells were collected, and measured cytokine A level by ELISA assay. (D) T and (E) NK cells were treated with supernatant, anti-A or cytokine A for 72 h, with IFN-γ level detected by ELISA assay.

Summary

Modifying OVs with immunomodulatory genes enhance its cytotoxicity on susceptible tumor cells compared to its mock counterpart, and significantly promotes the activation and proliferation of T, NK and moDC immune cells with increased IFN-γ secretion.