Oncolytic viruses (OVs), which preferentially infect cancer cells and induce host anti-tumor immune responses, have emerged as an effective melanoma therapy. Tanapoxvirus (TPV), which possesses a large genome and causes mild self-limiting disease in humans, is potentially an ideal OV candidate. The purposes of this study are to engineer TPV into effective OV candidates by arming immuno-stimulatory proteins and/or manipulating the virokines, and to explore the immuno-modulatory activities of TPV.

Interleukin-2 (IL-2) plays a critical role in activating T cells, natural killer cells and macrophages in both the innate and adaptive immune systems. In this study, a recombinant TPV expressing mouse IL-2 (TPVΔ66R/mIL-2) was generated, where the viral thymidine kinase (TK) gene (66R) was replaced with the mIL-2 transgene. We demonstrate that IL-2 inhibits virus replication through intracellular components and without activating the interferon-
signaling pathway. The anti-tumor potential of TPVΔ66R/mIL-2 was studied in athymic (T cell deficient) nude mice carrying human melanoma xenografts. Introduction of mIL-2 into TPV remarkably increased its anti-tumor activity, resulting in a more extensive cell degeneration with a significantly increased peri-tumor accumulation of mononuclear cells present in the tumors, compared with that in those treated with wtTPV or TPVΔ66R.

Neuregulin (NRG), an epidermal growth factor is known to promote the growth of various cell types, including human melanoma cells through ErbB family of tyrosine kinases receptors. TPV encoded protein TPV-15L, a functional mimic of NRG, also acts through ErbB receptors. We show that the TPV-15L protein promotes melanoma proliferation. TPV recombinant generated by deleting the 15L gene (TPVΔ15L) showed replication ability similar to that of wtTPV. Whereas, a TPV recombinant with both 15L and 66R genes ablated (TPVΔ15LΔ66R) replicated less efficiently than TPVΔ15L and the parental virus. TPVΔ15L exhibited more robust tumor-regression in the melanoma-bearing nude mice than other TPV recombinants.

Matrix metalloproteinases (MMPs), which are involved in degradation of extracellular matrix, are critical regulators in tumor metastasis. We report that infection with TPV promotes the expression of MMP-9 in the melanoma cells. In addition, we show that MMP-9 exerts an antiviral effect on TPV replication and plays a protective role in TPV-infected melanoma cells in vitro. Moreover, the neutralization of MMP-9 in melanoma cells remarkably enhances the TPV infection and leads to a significant reduction in cell survival.

In summary, our results suggest that 1) TPVΔ66R/mIL-2 is potentially therapeutic for human melanomas in the absence of T cells, and IL-2 expression results in an overall increase of therapeutic efficacy despite its viral inhibitory effects; 2) deletion of TPV-15L gene product which facilitates the growth of human melanoma cells can be an effective strategy to enhance the oncolytic potential of TPV for the treatment of melanoma; 3) in regards to the role played by MMP-9 in virus replication, identifying mechanisms that suppress MMP-9 expression upon TPV infection can potentially improve it used as a melanoma virotherapy.