Summary

Modulation of the tumor microenvironment represents a possible way to inhibit cancer growth and enhance anti-cancer immune responses. In the presented work we employ two strategies for tumor microenvironment modulation. Firstly, we have constructed rVACV co-expressing the tumor suppressor gene insulin-like growth factor-binding protein-3 (IGFBP-3) and the fusion gene encoding the immunogen SigE7LAMP. The expression of IGFBP-3 was regulated either by the early vaccinia virus H5 promoter or by the synthetic early/late (E/L) promoter. We have shown that expression of IGFBP-3 regulated by the H5 promoter yielded higher amounts of IGFBP-3 protein when compared with the E/L promoter. Immunization with P13-SigE7LAMP-H5-IGFBP-3 was more effective in inhibiting the growth of TC-1 tumors in mice and elicited a higher T-cell response against VACV-encoded antigens than the control virus P13-SigE7LAMP-TK$^-$. We found that high-level production of IGFBP-3 enhanced virus replication both *in vitro* and *in vivo*, resulting in profound antigen stimulation. Production of IGFBP-3 was associated with a higher adsorption rate of P13-SigE7LAMP-H5-IGFBP-3 to CV-1 cells when compared with P13-SigE7LAMP-TK$^-$. We have identified two structural differences between the IMVs of the IGFBP-3 expressing virus P13-SigE7LAMP-H5-IGFBP-3 and P13-SigE7LAMP-TK$^-$. The P13-SigE7LAMP-H5-IGFBP-3 IMVs incorporate the IGFBP-3 protein and they have elevated phosphatidylserine exposure on the outer membrane that could possibly result in increased uptake of these IMVs via macropinocytosis. The PS content of IMVs was measured by flow cytometry using microbeads covered with immobilized purified VACV virions.

Secondly, we have developed a DNA vaccine against the asparaginyl endopeptidase legumain that is overexpressed in M2-polarized TAM. To enhance the efficacy of DNA immunization against legumain, we performed several modifications of the legumain protein. These include mutagenesis of the RGD motif that resulted in diminished maturation and changed cellular localization of the legumain protein. Furthermore we inserted the p30 helper epitope from the tetanus toxin, which is capable to induce CD4$^+$ helper T-cells. Both modifications significantly enhanced the immune response against legumain. There was no further significant enhancement of the anti-legumain response when RGD mutation and p30 insertion were combined. DNA vaccination induces both T_H1 and T_H2 responses, of which only T_H1 promotes the induction of anti-tumor CD8$^+$ CTL. To enhance T_H1 and CTL responses we used CpG-ODN as adjuvant or depleted Treg using an antibody against CD25. Although administration of CpG-ODN showed no effect, the depletion of Treg significantly enhanced the immune response elicited by LgmnRGG.TT1l. We were able to show that immunization using LgmnRGG.TT1l was capable to significantly inhibit tumor growth, but did not inhibit formation of pulmonary metastasis nor altered the amount of TAM.