Enhanced gene therapy with TRAIL by bispecific antibody immuno therapy in advanced prostate and pancreatic cancer

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Introduction

Patients with advanced pancreatic or prostate cancer have a poor survival rate and new therapeutic strategies are needed. The overall aim of the project is the establishment and functional analysis of a new approach for the treatment of advanced prostate and pancreatic cancer. This approach will utilize a combination of the death ligand TRAIL as a pro-apoptotic agent over-expressed by T-cells and bispecific antibodies (bsAbs) targeting EpCAM on tumor cells and CD3 T-cells. The novel aspect is the combination of genetic modification with immune therapy. The lentiviral vectors allow the modification of lymphocytes to over-express the death ligand TRAIL (TNF-related apoptosis inducing ligand). The bispecific antibodies (bsAbs) binds with one arm the T cell via CD3 and with the second arm the tumor surface antigen EpCAM, thereby bringing tumor and effector cell in closer contact. We analyzed these features in established primary prostate (PC-3) and pancreatic (BxPC-3) carcinoma cell lines, in vitro three-dimensional tumor reconstructs and in mouse xenografts.

Transduction of PBMCs with lentiviral TRAIL

PBMCs were isolated from fresh healthy donor’s blood, and following activation with IL-2 and OKT3 were transduced with lentiviral TRAIL. The bispecific antibodies (bsAbs) binds with one arm the T cell via CD3 and with the second arm the tumor surface antigen EpCAM, thereby bringing tumor and effector cell in closer contact.

Apopotosis induction in 3D tumor reconstructs

To evaluate the efficacy of bsAbs combining EpCAMxCD3 antibody, we established an in vitro 3D tumor reconstruct system. To mimic tumor microenvironment, a mix of transduced lymphocytes were co-cultured with tumour cells and fibroblasts in collagen type I gel. Activation of lymphocytes was analyzed by secretion of IFN-γ and TNF-α (data not shown).

Results & Conclusion

We evaluated the anti-tumour efficacy of bispecific EpCAMxCD3 antibody linking tumour cells and T-lymphocytes. In NOD SCID mice, EpCAMxCD3 had a long serum half-life (11/2 = 7 days). EpCAMxCD3 significantly reduced growth of BxPC3 pancreatic and PC-3 prostate cancer xenografts. Since little apoptosis could be detected in these tumours on day 23 (TUNEL, Caspase 3), but lymphocytes (CD45+) could be detected – we assumed, that the time of observation for the mechanism resulting in the growth retardation and the cyst formation, was an earlier event. To further investigate the potential mechanisms of in vivo anti-tumour effects of EpCAMxCD3, we used a collagen gel 3D tumour reconstruct system, which closely resembled the tumour microenvironment. Therefore, to mimic the tumour situation, a mix of TRAIL transduced lymphocytes was co-cultured together with tumour cells and fibroblasts in collagen type I gel in vitro. In this setting apoptosis related proteins were expressed indicating an apoptosis induction in this system. To further prove the anti-tumour effect of the TRAIL-lymphocytes with the bsAb EpCAMxCD3, we performed proliferation assays incubating tumour cells with the bsAb loaded TRAIL-lymphocytes (data in preparation). With the previously demonstrated prolonged contacts between lymphocytes and tumor cells during the bsAb EpCAMxCD3, we could demonstrate a direct apoptosis effect through TRAIL-lymphocytes and bsAbs in vitro to explain the observed anti-tumour effects in vivo.

Overall, we provide a new combinatorial approach in which bsAbs targeting EpCAM on the surface of tumour cells and genetically modified lymphocytes over expressing TRAIL could improve the therapy in advanced prostate and pancreatic cancer patients.