Abstract to Reinhard Waehler's doctoral thesis

Adenoviral immunotherapy of solid tumors employing an HCC rat model

Generation of antitumor immunity by adenoviral gene transfer employing immunostimulatory genes is one of the most promising concepts in cancer gene therapy. Interleukins and T-cell costimulators have proven effective in various animal tumor models. The approach in this study is to provide (i) maximum immunostimulation by combined expression of single chain IL12 (scIL12), 4-1BBL and IL2 in one vector and (ii) minimal systemic side effects by intratumoral administration of a low viral dose. To accommodate all three genes in a single vector, a tricistronic expression cassette was constructed, linking the three cDNAs by two internal ribosomal entry sites (IRES). This adenoviral vector is termed Ad-3 (Ad-3: [PhCMV] scIL12 [IRES] 4-1BBL [IRES] IL2). Ad-3 expresses three independent proteins from one mRNA utilizing one hCMV-promoter. To control the effects contributed by 4-1BBL and IL2, Ad-1 ([PhCMV] scIL12) and Ad-2 ([PhCMV] scIL12 [IRES] 4-1BBL) were constructed. Gene expression was verified *in vitro* using ELISA for IL12 and IL2 and flow cytometry analysis for 4-1BBL. For *in vivo* application doses of Ad-1, Ad-2 and Ad-3 were adjusted to yield equal IL12 expression in cell culture.

Vectors were tested for their ability to eliminate tumor lesions in a syngeneic rat model of hepatocellular carcinoma. An effective vector dose was determined by injecting 1×10^7, 1×10^8 and 1×10^9 infectious units of Ad-3 in transplanted hepatic tumors. Magnetic resonance imaging (MRI) revealed dose-dependent tumor reduction. Long term antitumor effects of all vectors were determined at 5×10^6 and 5×10^7 infectious units per animal. Two tumor nodules were inoculated into the liver. Only one of those was treated to score for local and distal effects. Tumor volumes were measured by MRI 0, 3, 7 and 13 weeks after vector injection. Whereas none of the animals in the control group survived beyond week 7, 75-100% of animals in the treatment groups had no detectable tumors by week 13. Tumor-free animals that were treated with 5×10^7 infectious units were rechallenged with tumor cells and all of them eliminated the newly injected tumor cells, showing persistent antitumor immunity.

The dose of 5×10^6 infectious units is approximately 1000 times lower than the one used in an almost identical animal model for adenoviral IL12 therapy where 60% of the animals showed long term survival (Barajas 2001).

The results show the high efficacy of the newly constructed vectors at doses which may be considered as safe even when translated to men.