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New Strategies for the Therapy of Sarcomas Using Recombinant Adeno-associated Virus 2 Vectors that Contain a Suicide Gene

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Adeno-associated virus 2 (AAV-2) vectors are used for clinical gene therapy of hereditary diseases and at this stage for preclinical cancer gene therapy. In this work a high susceptibility of some solid tumors to an rAAV-2 vector containing the *humanized green fluorescent protein* (*hGFP*) gene was shown. Among a series of primary cells and eight solid tumor cell lines infected with these AAV-2 supernatants, the highest infection rates (functional titer: 200 IU/cell) were observed in soft tissue sarcoma cells (HS1; mean: 96% $hGFP^+$ cells) and in breast cancer cells (T47D; mean: 83%, MCF-7; mean: 85%); other cell lines (1 ovarian tumor, 1 germ cell tumor, 1 osteosarcoma and 2 small cell lung cancer) were less permissive (3% - 12%); $CD34^+$ peripheral blood progenitor cells showed the lowest transduction rates (max. 4%). These data suggest that the sarcoma and breast cancer cells are the most suitable candidates for further development of AAV-2 tumor suicide gene therapy. The first requirement for using these vectors in cell kill experiments was the construction of new rAAV-2 vectors containing a suitable suicide gene. The *thymidine kinase* (*TK*) gene was chosen in combination with the prodrug ganciclovir. Three novel *TK*-containing vectors were cloned. Since some of the new vectors contained no marker gene (*GFP*) and therefore did not allow fluorescence-based titration, a highly optimized titration method was established based on the real-time quantitative polymerase chain reaction so that rAAV-TK vectors which did not contain a fluorescent marker gene could be titrated accurately. Four sarcoma cell lines were chosen (HS-1, HT1080, RDES and SK-N-MC) and a complete eradication of all rAAV-TK/eGFP transduced tumor cells was shown following exposure to ganciclovir (2.5 µg/ml) *in vitro* while at this dose level >95% of mock-transduced tumor cells survived. Xenotransplantation tumor models for these human sarcoma cell lines were established. In proof of principle experiments mice transplanted with rAAV-TK/eGFP-transduced and ganciclovir-exposed tumor cells survived >5 months while in the non-transduced group all mice had died 1 month after inoculation. These data hold promise for a future clinical application of AAV-2-based suicide gene therapy.