Olijslagers Sharon
Dr. sc.hum.

Gene therapeutic application of the Chicken Anemia Virus-derived protein Apoptin

Date and birth place	23-07-1969, Boxtel, The Netherlands
A levels	1989
Studies	Plant breeding and crop protection from 1989 to 1995 Molecular Biology from 1995 to 2002
Practical training	in The Netherlands, Vancouver (Canada), and Heidelberg
Graduation	21/08/1995, Agricultural University, Wageningen, The Netherlands
Subject	DKFZ-ATV
Tutor	Prof. Jean Rommelaere

Apoptin, a chicken anemia virus protein, can specifically induce apoptosis in tumor or transformed cells, whereas other anti-cancer agents are not specific, clearly making Apoptin a promising agent for anti-tumor therapy. One can envisage two goals: firstly, the determination of new targets for anti-tumor therapy by unraveling the mechanism of Apoptin-induced apoptosis, and secondly, the development of gene therapy against cancer based on viral or other vectors expressing Apoptin. In this thesis, the focus is on the development of novel Apoptin-expressing vectors as well as the study of the effect of combined treatment with Apoptin and chemotherapeutic agents.

Part I

The oncotropic and oncolytic behavior of certain autonomous parvoviruses make them promising vectors for anti-cancer gene therapies. With the aim of enhancing the intrinsic antitumor effect of natural parvoviruses, a recombinant H1 virus vector was constructed which produces both the cytotoxic parvovirus regulatory protein NS1, as well as, the chicken anemia virus protein Apoptin, a protein endowed with tumor cell-specific apoptotic activities. The cytocidal activity exerted by the recombinant hH1/Apoptin virus was stronger than that of the green fluorescent protein (GFP)-transducing recombinant control virus hH1/GFP in three human tumor cell lines, which differed in their susceptibility to parental parvovirus H1-induced killing. Recombinant parvovirus-induced cytotoxicity was determined by nuclear staining with 4,6-diamidino-2-phenylindole (DAPI) of cells positive both for NS1 and GFP or Apoptin. Infection of these tumor cell lines with the Apoptin-transducing virus caused the death of almost all Apoptin-positive tumor cells irrespective of their wild-type p53 status or their over-expression of functional Bcl-2, even at low multiplicities of infection. The added value of Apoptin for vector-induced tumor cell killing was most pronounced in cells that were rather resistant to the genuine cytotoxic effect of the hH1 control virus. In contrast, hH1/Apoptin virus caused negligible cytotoxicity in normal human fibroblasts, comparable to the low death-rate of wild-type virus infection. Thus, it may be possible to reinforce the antineoplastic potential of autonomous parvoviruses in tumor cells through the construction of vectors that produce an additional toxic substance such as Apoptin, allowing the killing of a broader spectrum of tumor cells.

Part II

I also aimed at improving the cell-killing potential of plasmid- and adenovirus-directed Apoptin therapies by combining them with a chemotherapeutic agent. First, the cytotoxic range of various amounts of Apoptin was measured. The percentage of killed cells positively correlated with the amount of pCMVVP3 DNA transfected into U2OS cells, which in turn correlated with the net amount of Apoptin protein in the cells. At certain concentrations of transfected DNA encoding Apoptin and certain doses of Taxol treatment, the combination induced a greater, sometimes complete, reduction in colony viability than either treatment alone. A combination of Apoptin-expressing adenovirus AdAptVP3 infection with various chemotherapeutic agents such as MTX, Etoposide and Taxol all resulted all in an enhanced of the cytotoxicity of Apoptin in various tested human tumor cells. Under the conditions used, however, Taxotere did not. Finally, I have carried out some initial experiments to determine the underlying mechanism of the additional cell death effect observed with several cytotoxic agents on pCMVVP3 DNA or AptVP3-induced apoptosis. Taxol was proven to enhance the detected levels of Apoptin protein synthesized by transfected pCMVVP3 DNA, which positively influenced the overall cytoxicity. The chemotherapeutic agents Etoposide and MTX also seemed to enhance also the Apoptin-induced cytotoxicity via increase of the total level of Apoptin protein per cell. In conclusion, my results demonstrate that combining different Apoptin-expressing vectors with various chemotherapeutic treatments can increase Apoptin cytotoxicity.