Adenovirus-mediated Thymidine Kinase Gene Therapy Induces Apoptosis in Human Epithelial Ovarian Cancer Cells and Damages PARP-1

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Abstract. Adenoviral (ADV) gene therapy with the thymidine kinase gene (TK) under control of the Rous sarcoma virus (RSV) promoter followed by the administration of acyclovir leads to replication errors in transcription and to cell death. This concept of ADV-RSV-TK has been established for the treatment of ovarian cancer cells. The purpose of this investigation was to clarify whether cell death after ADV-RSV-TK gene therapy and acyclovir administration is indeed due to apoptosis induction, whether the synergistic effect of ADV-RSV-TK gene therapy with chemotherapy was limited to the primary mechanism of action or whether the vector transduction itself exerted any pro-apoptotic effect was examined using the epithelial cell lines OVCAR-3 and MDAH-2774, established from human poorly differentiated serous ovarian cancer. Fluorimetric assay of caspase-3 activity was performed, as well as ELISA of the CK 18 split product M30. PARP cleavage was analysed by Western blotting. Apoptosis induction was established in this investigation as the mechanism of the ADV-RSV-TK gene therapy effect of acyclovir administration by caspase activity and subsequent CK 18 cleavage. Neither acyclovir nor vector administration alone showed any apoptotic activity. The synergistic effect of TK gene therapy and chemotherapeutic agents was shown to be TK induced. Significant anti-PARP 1 activity was found to be an ADV-RSV-TK treatment effect after acyclovir addition.

ADV-RSV-TK gene therapy in combination with acyclovir administration may in the future play a role in the treatment of BRCA1- and 2-related familial ovarian cancer.

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persistent SSBs in DNA which result in formation of double-strand breaks (DSBs) when they meet at replication forks. In the event of DNA damage associated with PARP inhibition, specifically \textit{BRCA1} or \textit{BRCA2} mutant cells cannot repair DNA DSBs properly, leading to the collapse of replication forks. Thereby abnormal DNA ends join and consequently lead to growth arrest and apoptosis. This model provides a molecular basis for the observation that \textit{BRCA1}- and \textit{BRCA2}-deficient cells are extremely sensitive to PARP-1 inhibition.

The purpose of this investigation was to clarify whether cell death after ADV-RSV-TK gene therapy and acyclovir administration is indeed due to apoptosis induction. It was also of interest to clarify whether the synergistic effect of ADV-RSV-TK gene therapy and chemotherapy (6) was limited to the primary mechanism of action, or whether the vector transduction itself exerted any pro-apoptotic effect. As ovarian cancer is part of the \textit{BRCA1} and 2 related inheritable cancer syndromes, the effect of \textit{TK} gene therapy on PARP-1 function may be of special interest (7).
Materials and Methods

Fluorimetric assay of caspase-3 activity was performed according to the instructions of the manufacturer (Promega, Madison, Wisconsin, USA).

An enzyme-linked immunosorbent assay (ELISA) was used to measure M30-antigen levels (Apotosense, DiaPharma, West Chester, Ohio, USA).

Mouse anti-human PARP-1 antibody (ABR affinity, Golden, Colorado, USA) was used for Western blot analysis of PARP cleavage.

The human epithelial ovarian cancer cell lines MDAH-2774 and OVCAR-3 (cell lines graciously provided by L.A. Jones, Ph.D., M.D. Anderson Cancer Center, Houston, Texas) were grown to 80% confluency following standard published protocols (1). The green fluorescent protein (GFP) gene was incorporated into the adenoviral vector and used to test the influence of adenoviral transformation on the sensitivity of these cells to topotecan chemotherapy. The 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT)-based assay was used to quantify cell viability during treatment with topotecan chemotherapy and ADV-GFP (8, 9). Susceptibility to chemotherapy- and vector-induced cell killing was compared at a multiplicity of infection (MOI) of 66 established in previous experiments (10).

All assays were run in duplicate.
Results

Apoptosis induction in MDAH-2774 and OVCAR-3 human epithelial ovarian cancer cells became evident three days after ADV-RSV-TK transduction and acyclovir substrate addition. Caspase-3 activity was maximal after three days and showed a decrease on days 4 and 5 (Figure 1).

Cytokeratin 18 cleavage, measured by the M30 assay, by contrast, gradually increased from day 3 to 5 in OVCAR-3 cells, while peak levels were observed in MDAH-2774 cells on day 4 with a subsequent decline (Figure 2).

PARP cleavage analysis showed the same pattern and differential behaviour in both cell lines, with PARP cleavage increasing continuously until day 5 in OVCAR-3 cells while decreasing after day 3 in MDAH-2774 cells (Figure 3).

Neither acyclovir nor vector administration alone showed any apoptotic activity. The synergistic effect of TK gene therapy and chemotherapeutic agents was equally shown to be TK induced (Figure 4), as vector alone did not provide any additive antitumor activity.

Discussion

The presented results are preliminary.

Apoptosis induction was established in this investigation by caspase activity and subsequent CK 18 cleavage as the mechanism of the ADV-RSV-TK-induced gene therapy effect after acyclovir administration. ADV-RSV-TK gene therapy in combination with acyclovir administration has been shown to be well tolerated as a single intraperitoneal treatment course in patients with recurrent ovarian cancer in combination with topotecan chemotherapy and 2.5-year results can be interpreted as encouraging (11). As neither the vector nor the substrate by itself exerted any apoptotic effects, the postulated dose-dependent and substrate-limited character of this treatment can be considered as established. In view of its significant anti-PARP activity, gene therapy of ovarian cancer with this gene therapy concept should certainly also include cases of BRCA1- and 2-related cancer. One might even speculate that this type of therapeutic mechanism should be tested specifically in a subset of patients with familial breast-ovarian cancer syndrome, as it might realise its maximum potential under these genetic conditions.

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References


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