

Persistent Adenovirus-mediated *Thymidine Kinase* Gene Expression in Ovarian Cancer Cells Increases Cell Killing Efficacy Over Time

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Abstract. Adenovirus (ADV)-mediated gene therapy with the thymidine kinase (TK) gene under control of the Rous sarcoma virus (RSV) promotor followed by the administration of acyclovir has been established *in vitro* for the treatment of ovarian cancer cells and has been used as the basis for intraperitoneal phase I clinical trials. It is unclear how long a significant degree of transgene translation can be expected after adenovirus-mediated TK transduction, where the transcriptional complex is localized in the nucleus in an episomal fashion and thus without stable integration. The possible interaction of acyclovir pretreatment with subsequent ADV-RSV-TK transduction also remains to be elucidated. Transgene expression and cell killing efficacy were analysed based on multiplicity of infection (MOI) and MTT assay. Anti-TK-antibody 1397 was used for immunocytochemistry and Western blot analysis of TK expression. After transduction with ADV-RSV-TK at an MOI of 66, TK translation increased strongly in MDH 2774 and OVCAR-3 cell lines during the initial 48 hours. Virtually constant expression of the TK transgene was observed by Western blot during eight days. Cell killing efficacy was increased by repeated daily administrations of acyclovir. Pretreatment with acyclovir did not result in significantly increased cell killing efficacy. No negative effect of acyclovir on ADV-RSV-TK transduction was observed. The at least week-long expression of the TK transgene with persistently increasing efficacy of cell killing after ADV-mediated tumor cell transduction provide a realistic basis for the development of multicycle ADV-mediated TK gene therapy

approaches in the treatment of ovarian cancer. Continuous *i.v.* acyclovir treatment or daily oral acyclovir-prodrug therapy might simplify the substrate regimen for the TK gene.

Adenovirus (ADV)-mediated gene therapy with the thymidine kinase (TK) gene under control of the Rous sarcoma virus (RSV) promotor followed by the administration of acyclovir leads to replication errors in transcription and to cell death. This concept has been established *in vitro* for the treatment of ovarian cancer cells (1) and has been used as the basis for intraperitoneal phase I clinical trials also in combination with chemotherapy (2). In these investigations, one cycle of therapy has been tested and has been found to be well tolerated.

Recently, we reported that expression of the coxackie adenovirus receptor (CAR) persists in adenovirus-transduced cells. This would make repeated ADV-mediated gene transfer possible (3).

It is unclear how long a significant degree of transgene translation can be expected after ADV-mediated TK transduction, where the transcriptional complex is localized in the nucleus in an episomal fashion and thus without stable integration. The possible interaction of acyclovir pretreatment with subsequent ADV-RSV-TK transduction also remains to be elucidated. The answer to these questions is important when designing the duration of acyclovir administration during future multicycle ADV-mediated TK gene therapy trials.

Materials and Methods

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).

Anti-TK-antibody 1397 was used for immunocytochemistry and Western Blot analysis of TK expression in the transduced ovarian cancer cells. Antibody production and specificity were previously published (3).

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Key Words: Gene therapy, thymidine kinase, adenovirus, coxackie-adenovirus receptor, ovarian cancer

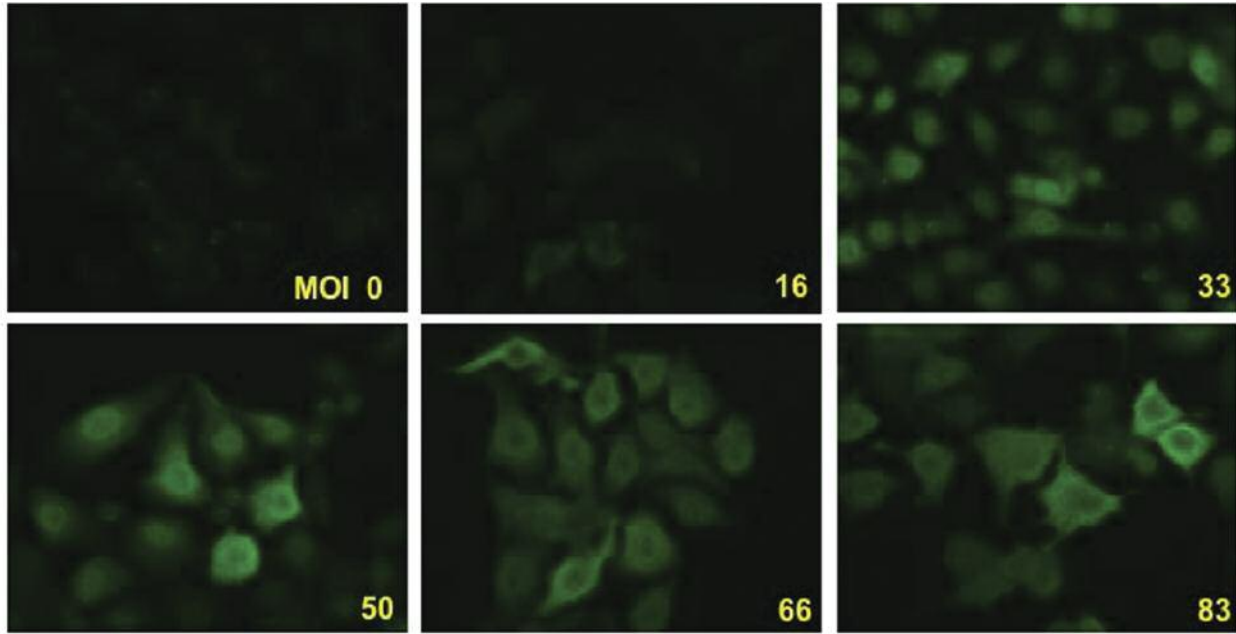


Figure 1. Thymidine kinase expression in MDAH-2774 epithelial ovarian cancer cells 24 hours after transduction with different MOI of ADV-RSV-TK.

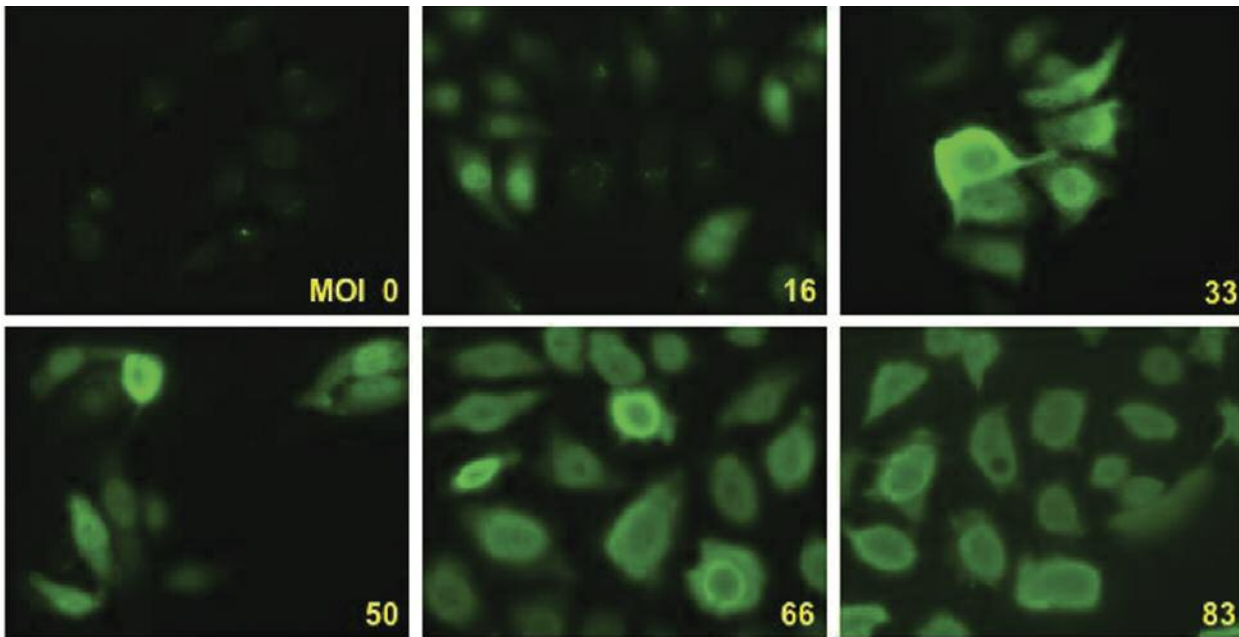


Figure 2. Thymidine kinase expression in OVCAR-3 epithelial ovarian cancer cells 48 hours after transduction with different MOI of ADV-RSV-TK.

Transgene expression and cell killing efficacy were analysed based on a uniform multiplicity of infection (MOI) of 66 inducing 100% of the cells to produce TK after 24 hours (MDAH-2774), or 48 hours (OVCAR-3) (Figures 1 and 2). Western blot confirmed maximum TK translation at this MOI (Figure 3).

In order to investigate if faster cell-killing was achieved when acyclovir treatment was started after transduction, this approach was compared to cellular transduction with acyclovir already present.

The design for the experiment addressing the possible interaction between cellular acyclovir pretreatment and ADV-RSV-TK transduction

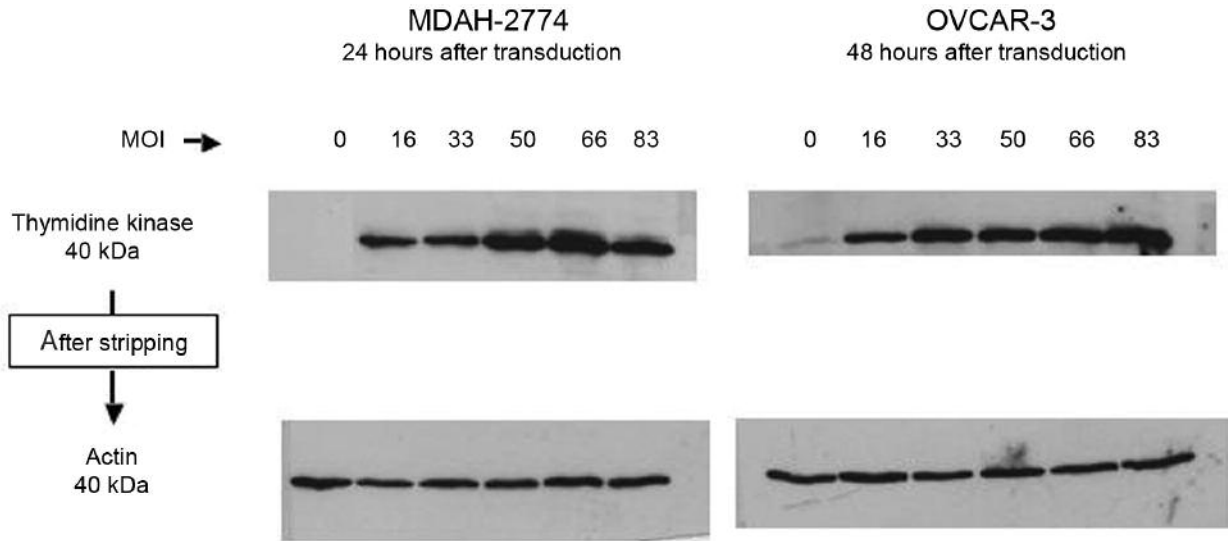


Figure 3. Western blot of thymidine kinase expression after transduction with different MOI of ADV-RSV-TK.

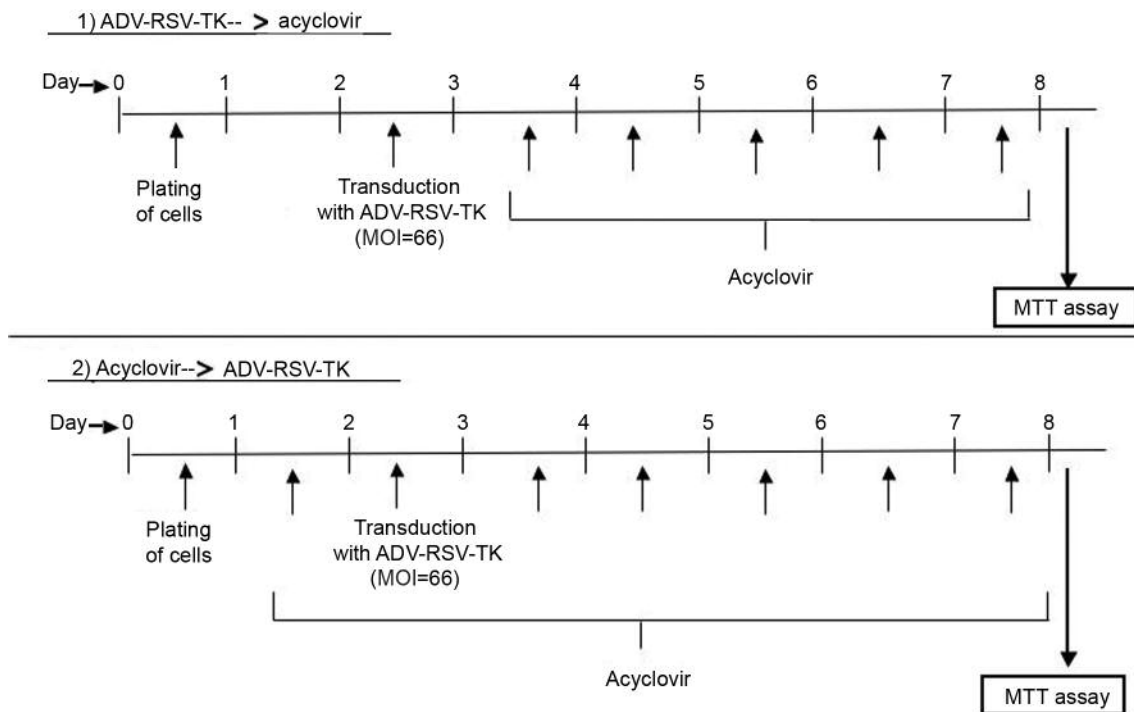


Figure 4. Experimental design for investigation of the interaction between acyclovir treatment and ADV-RSV-TK transduction.

is shown in Figure 4. MDAH-2774 cells were plated at 50,000 cells/well and OVCAR-3 cells at 30,000 cells/ well in 24 well-plates. In the cell killing experiments, cells were treated with acyclovir at a concentration of 150 mg/ml (MDAH 2774) and 50 mg/ml (OVCAR-3). The acyclovir/RPMI-1640 solution was prepared fresh every day.

On days 3, 4, 5 and 6, one partial set of the experiments was stopped and a 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium

bromide (MTT) assay was performed. The MTT-based assay was used to quantify cell viability during treatment with ADV-RSV-TK gene therapy (4, 5).

The cells were incubated with 0.25 mg MTT/ml RPMI for 1 hour. The medium was aspirated and the residue was dissolved in 1 ml DMSO. Then 100 ml were measured in a 96-well plate at 595 nm. All assays were run in duplicate.

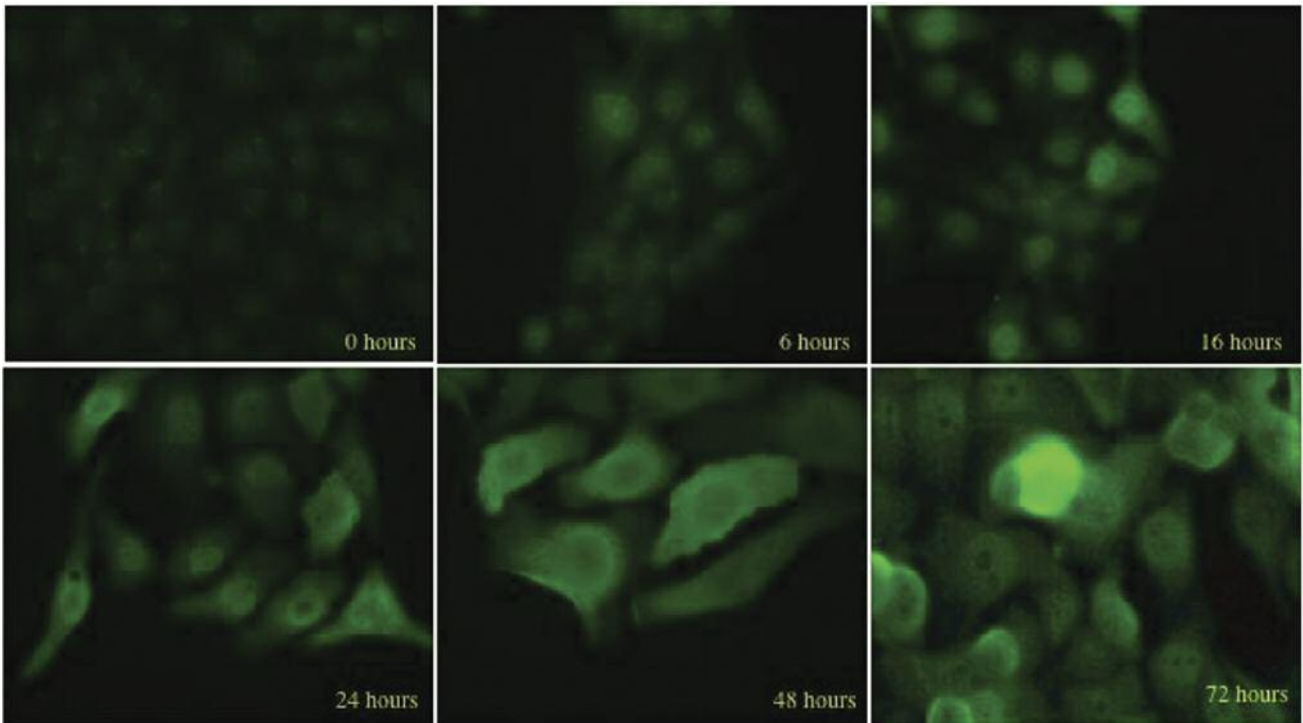


Figure 5. Time-dependent thymidine kinase expression in MDAH-2774 human epithelial ovarian cancer cells after transduction with ADV-RSV-TK (moi=66).

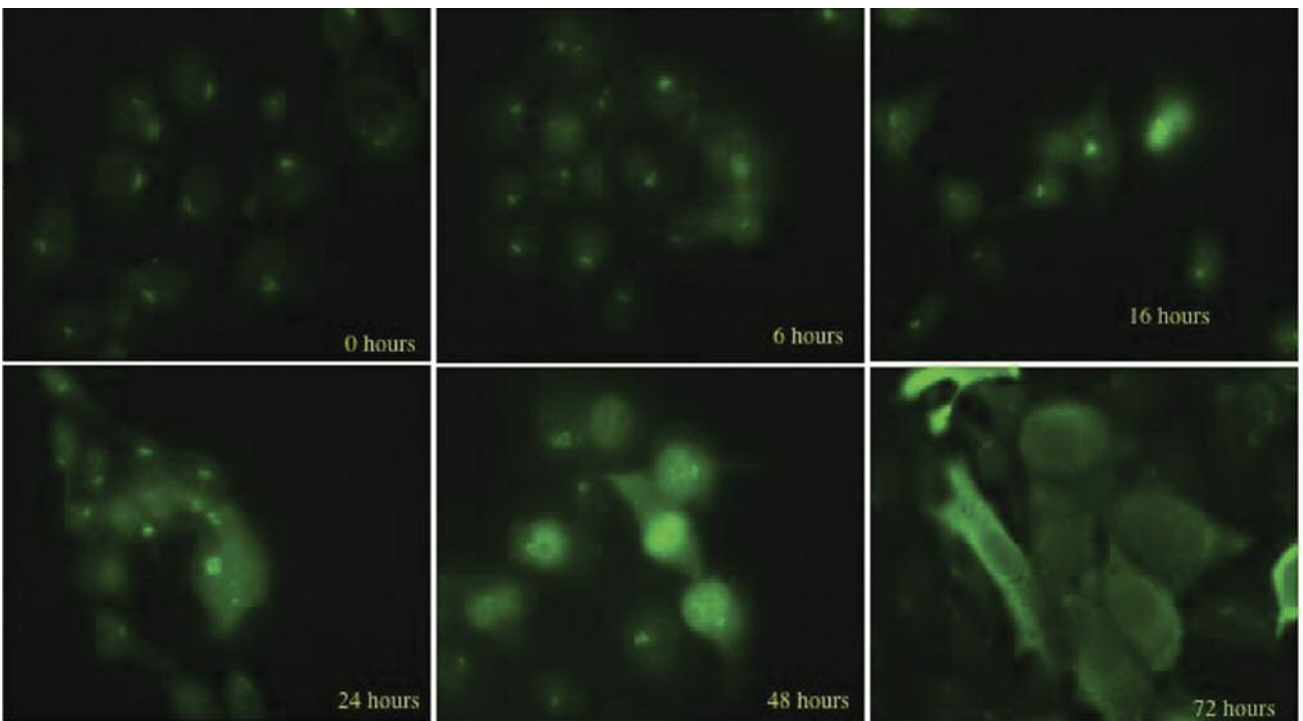


Figure 6. Time-dependent thymidine kinase expression in OVCER-3 human epithelial ovarian cancer cells after transduction with ADV-RSV-TK (moi=66).

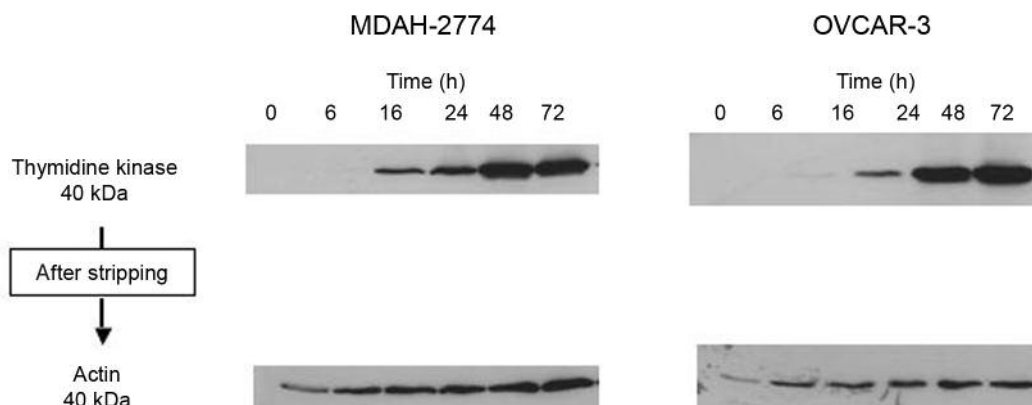


Figure 7. Western blot of time-dependent thymidine kinase expression after transduction with ADV-RSV-TK (MOI=66).

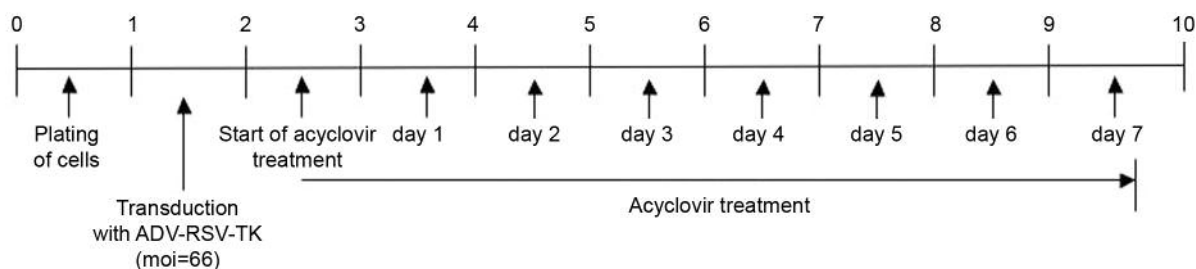


Figure 8. Time course of prolonged expression experiment.

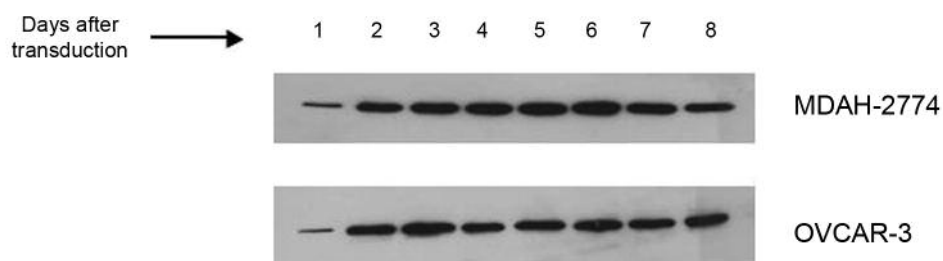


Figure 9. Time-dependent thymidine kinase protein expression in ovarian cancer cell lines after transduction with ADV-RSV-TK (MOI=66) and without acyclovir administration.

Results

After transduction with ADV-RSV-TK at an MOI of 66, TK translation increased strongly in both cell lines during the initial 48 hours as shown by immunocytochemistry (Figure 5, 6) as well as by Western blotting (Figure 7).

The experimental design of the long-term expression experiment is depicted in Figure 8.

When investigating TK gene expression during eight days without addition of acyclovir and thus without cell

killing, a virtually constant expression of the TK transgene was observed by Western blot (Figure 9). Interexperimental variability was minimal as indicated by the error bars.

In concordance with the expression pattern, cell killing efficacy was increased by the repeated administrations of acyclovir (Figure 10).

While the response curve was different between the two cell lines, after 7 days a reduction of surviving tumor cells by 80% was observed. The presence of surviving cells was probably due to the phenomenon that likely not 100% of the

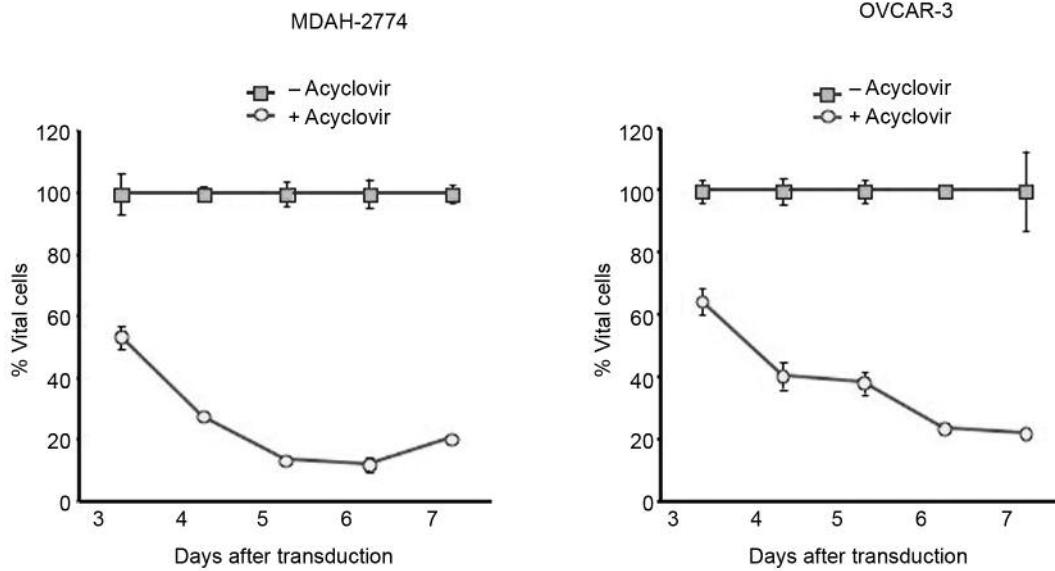


Figure 10. Time kinetics of cell death in ovarian cancer cell lines after transduction with ADV-RSV-TK (MOI=66) and treatment with or without acyclovir.

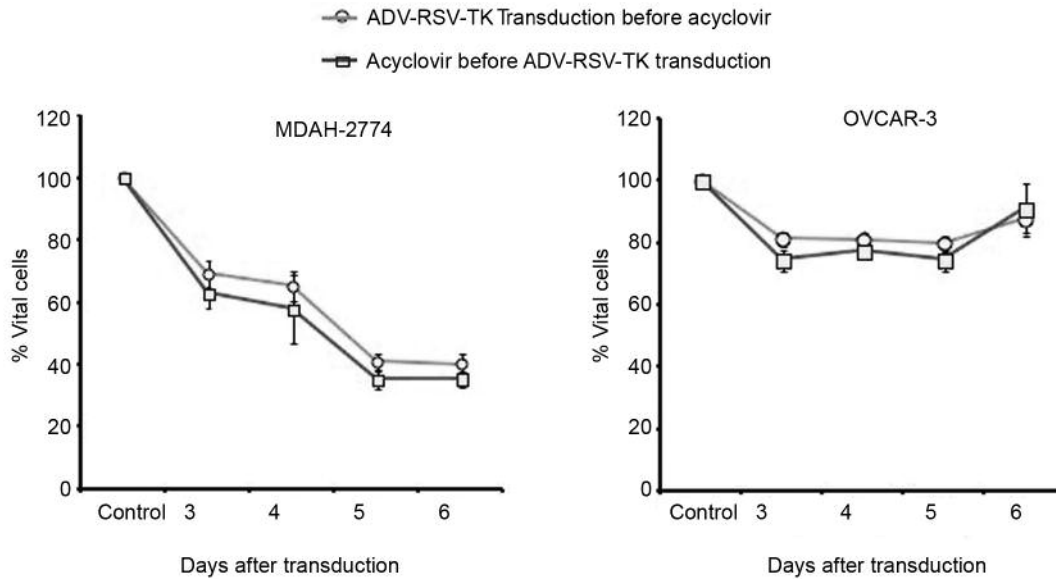


Figure 11. Influence of timing of acyclovir administration on cell killing efficacy.

plated cells had been initially transduced and thus a certain amount of regrowth of untransduced cells unaffected by the bystander effect occurred over time.

Pretreatment with acyclovir did not result in a significantly increased cell-killing efficacy. On the other hand, no negative effect of acyclovir on ADV-RSV-TK transduction was observed (Figure 11).

Discussion

Intraperitoneal *TK* gene therapy of ovarian cancer may become a promising approach for a dose-dependent biological tumoricidal treatment concept with an additional potentially synergistic effect with chemotherapeutic agents (6). Persistent expression of CAR (3), as well as the at

least week-long expression of the TK transgene with persistently increasing efficacy of cell killing after ADV-mediated tumor cell transduction described here provide a realistic basis for the development of multicycle ADV-mediated TK gene therapy approaches in the treatment of this disease. One might speculate on the basis of the data presented that, in view of prolonged TK expression by transduced cells, the time interval between vector deliveries could well be synchronized with the usual 3-week interval of taxane-based combination chemotherapy, while continuous *i.v.* acyclovir treatment or daily oral acyclovir-prodrug therapy would not interfere with tumor cell transduction and might simplify the substrate regimen during TK gene therapy. As any prolonged TK activity will lead to an enhancement of the antitumor effect, continuous acyclovir would take maximum advantage of prolonged TK expression. Cell line studies frequently provide a useful approximation of the situation *in vivo*, specifically helping in devising an appropriate investigational strategy for the clinical setting. However, wherever ethically sustainable, tolerable and safe for the patient as well as technically feasible, parameters of gene therapy biology should be monitored as part of any clinical evaluation to facilitate further evolution of gene therapy concepts and ultimately to optimize prognosis for the patient with advanced ovarian cancer.

Acknowledgements

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