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Gene therapy of cancer: enhancement of gene expression and construction of tissue-specific vectors for C cell carcinoma

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For cancer gene therapy, the transfer and expression of suicide genes into malignant tumor cells represents an attractive approach. One of the main problems of this system is that in general, the *in vivo* transduction efficiency of most viral vectors including rAAV vectors is too low to achieve therapeutic levels of the suicide enzyme in the tumor cells. New approaches to obtain a sufficient level of enzyme activity in the tumor remain an ambitious goal.

Gemcitabine is metabolized in the cell into its active nucleotide forms, dFdCDP and dFdCTP, which cause DNA damage by competing with dCTP for incorporation into DNA and may lead to an enhancement of rAAV transduction. To verify this hypothesis, we constructed rAAV vectors containing the HSVtk, the EGFP or hNIS gene under the control of the CMV promoter and assessed the effects of gemcitabine treatment on the rAAV infection process and the expression of EGFP, HSVtk, and hNIS. The confocal microscopy demonstrated that pre-treatment of cells with gemcitabine does not significantly influence the binding of Cy3-conjugated AAV onto the cell membrane, internalization of the virus into the cells, redistribution of the virus in the cells, accumulation of the virus around the nuclear envelope and trafficking of the virus into the cell nucleus. The FACS analysis showed small changes in rAAV infection efficiency (5% ~ 10%). However, the EGFP expression was greatly enhanced in a dose-dependent manner after treatment of cells with gemcitabine. A dose-dependent increase of fluorescence activity was also observed under the digital fluorescence microscope. The fluorescence spectroscopy revealed that the EGFP activity was increased during 48 h p.i. and up to 20.4-fold after treatment of the cells with the IC_{50} dose of gemcitabine. For the suicide strategy of HSVtk-mediated cancer gene therapy, ^{3}H-GCV uptake of HeLa cells pretreated with an IC_{50} dose of gemcitabine followed by infected with rAAV/CMVtkneo was significantly increased to 2.42-fold of that without pretreatment with gemcitabine. Furthermore, gemcitabine treatment led to a 1.5-fold increase of ^{3}H-GCV uptake in a stable HeLa subline

transformed by infection with rAAV/CMVtkneo particles. The expression of the hNIS gene mediated by transient infection with rAAV induced a high uptake of radioiodide in HeLa cells which was comparable to that of the wild type rat follicular cell line FTRL-5. Pretreatment of HeLa cells with gemcitabine led to a 1.87-fold increase of radioiodide uptake. Enhancement of gene expression after treatment of cells with gemcitabine was also observed in other cells lines such as hMTC·DR cells and murine NIH3T3 cells. Employing EMSA analysis, the phosphorylated form of ssD-BP was observed in control and after treatment with tyrphostin 1, gemcitabine, 5-FU, adeno 5, H_2O_2, vanadate and pervanadate in both nuclear extracts immediately after drug treatment or at 24 h after treatment. The dephosphorylated form of ssD-BP was only observed after tyrphostin 51 treatment, but this dephosphorylated form was reversed to the phosphorylated form at 24 h after tyrphostin 51 treatment. Taken together, we conclude that pretreatment of cells with gemcitabine greatly enhances rAAV transduction in a dose-dependent manner, which is mainly caused by the increase of gene expression, but not by changes in the infection process or the phosphorylation state of ssD-BP.

To avoid side-effects and to gain high therapeutic efficacy in cancer gene therapy, a tissue-specifc gene expression is the most recommended strategy. In this study, we also showed that suicide gene expression in a tisue-specific manner was achieved in medullary thyroid carcinoma using calcitonin regulatory elements and was greatly enhanced by modification of these elements. To investigate the role of the CT gene regulatory sequences for tissue specific gene expression in MTC cells the CT promoter (CT266) region alone or with enhancer elements (CTenh) or the CMV promoter were cloned upstream the genes coding for the HSVtk and G418 resistance coupled by an IRES site leading to the rAAV constructs rAAV/CT266tkneo, rAAV/CTenhtkneo and rAAV/CMVtkneo. ^{3}H-GCV uptake experiments revealed HSVtk activity after infection with rAAV/CT266tkneo particles in the CT expressing TT and hMTC cells and low GCV accumulation in HeLa cells. cAMP treatment of TT cells increased the GCV uptake up to 20%. Upon GCV treatment a significant but less tissue-specific growth inhibition of TT cells transiently infected by rAAV/CT266tkneo particles (74.5% growth inhibition) and of the stable HSVtk expressing cell line TT/AAV/CT266tkneo-7 (55% growth inhibition) was observed while the proliferation of TT cells transiently infected by rAAV/CTenhtkneo (33.6% growth inhibition) or the stable cell line TT/AAV/CTenhtkneo-16 (38.1% growth inhibition) was effected less but more tissue-specific. To enhance the up-regulative activity of the CT regulatory elements, a modification of these elements was done by inserting 4 copies of EtsE2 between the CT promoter and 3 copies of the basal enhancer fragment. ^{3}H-GCV uptake in rMTC 6-23 cells infected with rAAV/3×CTenhEtsE2CT266HSVtk can be enhanced up to 17-fold of that in HeLa cells and 7-fold

to that in rMTC 6-23 cells infected with rAAV bearing the original CT regulatory element. The ^{3}H-thymidine incorporation in rMTC 6-23 cells infected with the rAAV and treated with GCV was decreased to only 2% of that without infection. From these results, we conclude that employing the original regulatory elements of the calcitonin gene, a tissue-specificity is achieved with low level of gene expression. Multienhancer fragments in the regulatory elements greatly enhance the gene expression and maintain the high tissue specificity.