

THE USE OF ADENOVIRUS IN GENE TRANSFER TO PANCREATIC TUMOUR CELL LINES

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ABSTRACT

Pancreatic cancer is often a fatal disease due to its poor response to existing therapies. Thus new therapeutic approaches must be developed. The aim of this study was to evaluate the susceptibility of a series of pancreatic cell lines to adenovirus-mediated transduction and to determine the expression of surface coxsackie B and adenovirus receptor (CAR). Eleven human pancreatic cancer cell lines (BxPc-3, NP9, PSN-1, Capan-1, PT45, ASPC-1, T3M4, MIACaPa-2, NP18, Panc-1, A818-4) were transduced using a recombinant adenovirus type 5 expressing enhanced green fluorescent protein (Ad5EGFP) and the expression of EGFP was analysed by flow cytometry. The cell lines varied in transduction efficiency, ranging from less than 1% to more than 30% cells expressing EGFP and susceptibility of cell lines to Ad5EGFP transduction has a positive correlation with the level of surface CAR expression and its presence may be a limiting step for efficient adenovirus transduction. These results suggest that gene therapy of pancreatic tumours is feasible, but then the status of CAR expression in the tumour needs to be evaluated.

KEYWORDS: Adenovirus; CAR; pancreatic tumour, gene therapy

INTRODUCTION

Adenovirus (Ad) vectors have been used widely in clinical trials for gene transfer, especially for the treatment of cystic fibrosis [1]. Recently, adenovirus gene therapy is being developed to treat cancer patients [2]. Since pancreatic cancer is a highly fatal disease with only 10% of patients surviving 5 years even when conventional treatment can be accomplished [3], the use of adenoviruses as therapeutic gene delivery systems may offer a new approach to the treatment of pancreatic tumours. However, this requires basic studies of the adenovirus entry process in pancreatic tumour cells.

The capability of an Ad vector to infect a cell depends crucially on the primary (CAR) and secondary adenovirus receptors (integrins) expression [4]. CAR expression levels seems to be a limiting step in Ad transduction of skeletal muscle, endothelial and smooth muscle cells, brain cells, bladder cancer cells, ovarian cancer [5], and human melanoma cells cultures [6].

In the present study, we have investigated the transduction of eleven pancreatic tumour cell lines by a recombinant adenovirus containing green fluorescent protein gene (Ad5EGFP). The level of surface CAR expression in pancreatic tumour cell lines has also been assessed.

We find a strong correlation between the level of surface CAR expression and the level of transduction of pancreatic tumour cell lines by Ad5EGFP. This suggests that, in future gene therapy studies of pancreatic cancer by adenoviruses, the level of CAR expression in the tumour needs to be assessed if adenovirus-mediated gene therapy is to be effective.

MATERIALS AND METHODS

Adenoviral vectors

Ad5EGFP virus is a replication-deficient recombinant Ad5 expressing the enhanced green fluorescent protein (EGFP) under the control of the cytomegalovirus (CMV) promoter, inserted in place of the E1 region.

Cell culture

All pancreatic and A549 cells were grown in DMEM and CHO cells were grown in MEM alpha medium and were maintained at 37°C in a 5% CO₂ atmosphere.

Flow cytometry analysis

Two hundred thousand cells were incubated with either 50 µl of rabbit anti-human CAR or no primary antibody with 50 µl fluorescein isothiocyanate-conjugated secondary antibody and analysed on a FACScalibur flow cytometer (Becton Dickinson, Oxford, UK).

Transduction of cells with Ad5EGFP

Cells (1×10^5) grown at approximately 80-90% confluency were treated with Ad5EGFP at a multiplicity of infection (MOI) of 100 focus forming unit (FFU) per cell and the EGFP expression was assessed by flow cytometry.

RESULTS

Transduction of pancreatic tumour cells by recombinant adenovirus expressing green fluorescent protein

Since the successfully transduced cells can be directly measured by the presence of the EGFP transgene, this enabled the determination of gene transfer efficiency without selection or antibody staining. Conversely, cells resistant to adenovirus-based transduction will not express the EGFP transgene product. CHO cells have been used as an example of CAR-deficient cells. A549 cells were used to represent CAR-expressing cells.

Figure 1 showed that certain cells were resistant to adenovirus transduction, whereas others were susceptible. Transduction efficiencies ranged from less than 1% to more than 30% of cells. PSN-1 cell lines showed strong resistance to transduction by Ad5EGFP (<1% transduction). In contrast, the Panc-1 cell line was most susceptible (35% transduction) to transduction by Ad5EGFP, followed by NP18, ASPC-1 and Capan-1. The control cell line, A549 showed the highest levels of transduction while CHO cells exhibited poor transduction efficiency. Low levels of EGFP expression in Ad5EGFP transduced pancreatic tumour cells suggests a reduced efficiency of gene transfer which could be due to a number of reasons, including impaired binding of virus at the plasma membrane. This was investigated further by assaying levels of surface receptors for adenoviruses in pancreatic tumour cells.

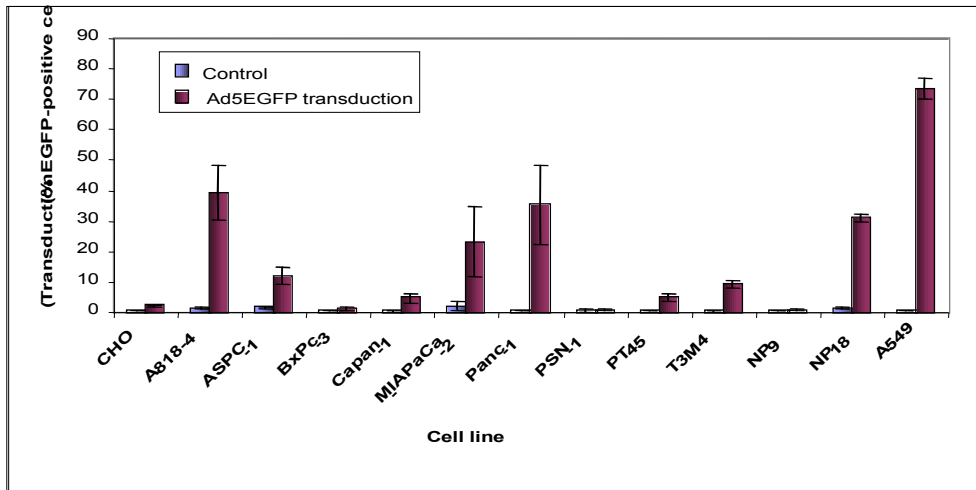


Figure 1. Ad5EGFP-mediated transduction in a panel of human pancreatic tumour cell lines.

Expression of the coxsackie B and adenovirus receptor (CAR) in pancreatic tumour cell lines

Eleven pancreatic tumour cell lines, CHO and A549 cells were analysed for cell surface expression of CAR by indirect immunofluorescence assay. Flow cytometric analysis of cell surface CAR expression (Figure 2) revealed that it was detectable in all of the pancreatic tumour cells.

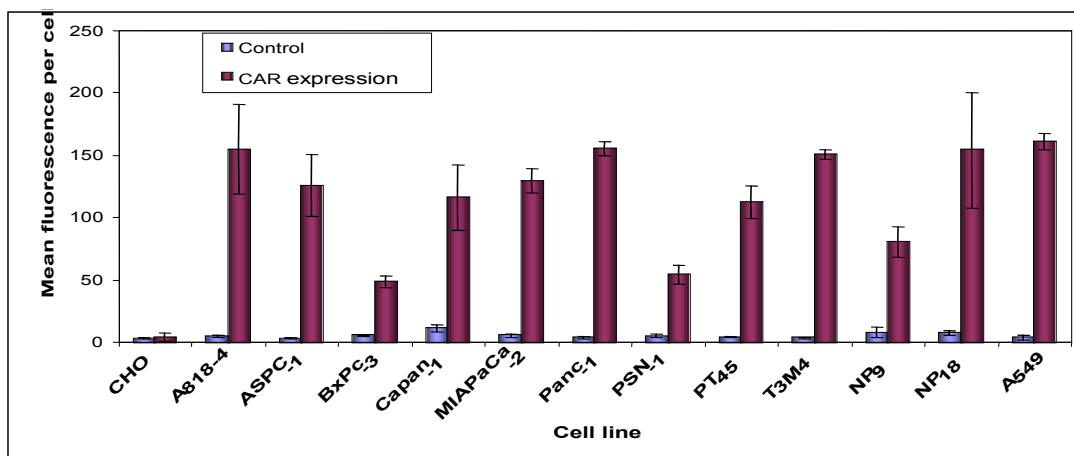


Figure 2. Expression of CAR cell surface protein in a panel of human pancreatic tumour cell lines.

However, expression of CAR varied between 50 to more than 150 mean fluorescence intensity (MFI). Panc-1 and NP 18 cells exhibited a relatively high CAR expression of more than 150 MFI per cells, which was comparable to A549 cells that formed the positive control (160 MFI per cell). In contrast, PSN-1 cells showed a rather low CAR expression of less than 60 MFI per cells. The remainder of the pancreatic tumour cell lines expressed intermediate levels of CAR on the cell surface. As expected, CHO cells had an almost no detectable surface CAR expression.

Correlation of CAR expression with adenovirus transduction of pancreatic tumour cell lines

To examine the correlation between the susceptibility of pancreatic tumour cell lines to transduction by Ad5EGFP and the expression level of surface CAR, the level of CAR expression versus the percentage of EGFP-positive cells was plotted (Figure 3) and Pearson's statistical analysis was performed using SPSS 11.0 software [7]. As shown in Figure 3, it seems likely that the lower susceptibility of pancreatic tumour cell lines to adenovirus transduction might be related to the differences in expression level of CAR, because the cell lines with the highest level of CAR demonstrated an increase susceptibility to transduction with adenovirus and those cell lines with the lowest levels of surface CAR were refractory to adenovirus transduction. These data indicated that the level of CAR on the cell surface is an important factor in the efficacy of gene transfer.

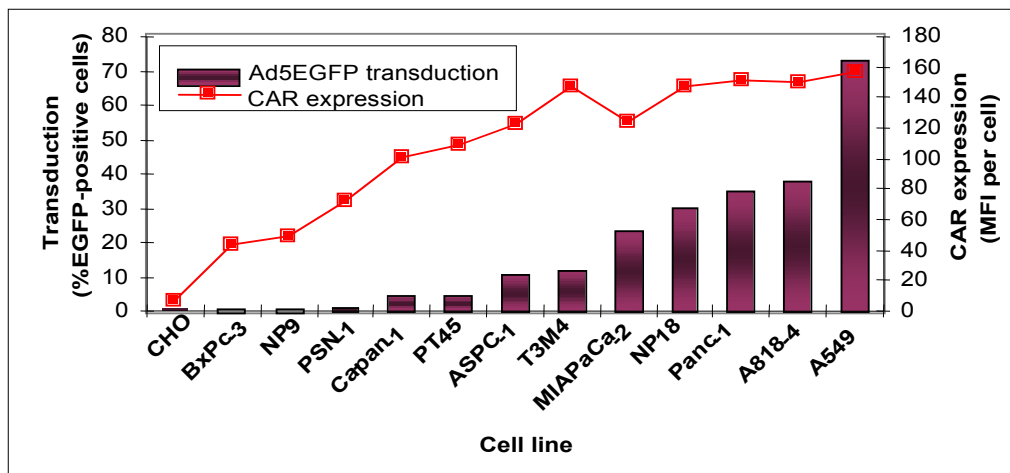


Figure 3. Relationship between Ad5EGFP-mediated transduction and CAR expression in a panel of human pancreatic tumour cell lines.

DISCUSSION

Adenovirus gene therapy is an intense area of research and is being developed as a therapeutic modality for a variety of malignancies [1]. Since pancreatic cancer shows a poor response to existing treatment, thus there is a great need of new approach to overcome this problem.

Therefore, study was performed to evaluate the susceptibility of a series of pancreatic cell lines to adenovirus-mediated transduction. It was found that all the pancreatic tumour cell lines were relatively susceptible to transduction by Ad5EGFP (Figure 1). However, PSN-1 pancreatic tumour cell lines exhibited strong degree of resistant to adenovirus-mediated transduction. Previous studies have also shown that certain tumour types and established cell lines, for example in human bone marrow cells [8] were relatively resistant to adenovirus-mediated gene transfer. Furthermore, transduction of Ad5EGFP showed a strong correlation ($p < 0.01$) between transduction and the level of CAR surface expression in each of the cell lines, as determined by flow cytometry (Figure 3). Clearly, efficient transduction of pancreatic tumour cell lines by Ad5EGFP requires adequate CAR expression.

We also showed that cell surface CAR was detected in all of the pancreatic tumour cell lines but varied in the level of expression, as shown by flow cytometry analysis (Figure 1). This lead to the fact that CAR is the primary receptor for Ad5 in pancreatic cells in culture, highest level of CAR demonstrated an increase susceptibility to transduction with adenovirus and those cell lines with the lowest levels of surface CAR were refractory to adenovirus.

CONCLUSION

The conclusion of this study is that gene therapy of pancreatic tumour cells is feasible using Ad-mediated gene transfer, and also suggesting that if adenovirus vectors are applied to pancreatic cells, the status of CAR expression in the tumour needs to be evaluated. In this respect, the particular treatment required for pancreatic cancer may need to be tailored to the individual patient.

ACKNOWLEDGMENTS

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REFERENCES

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