In their article, Haviv et al. (1) suggested that gene therapy for renal cancer with the conventional serotype 5 adenoviral vectors is limited due to a deficiency in expression of the CAR 1 on RCC cells. Subsequently, they suggested and described the use of alternative adenoviral vectors targeted to the subgroup B adenovirus receptor or to the αv integrins more abundantly expressed on RCC cells. Unfortunately, their study was only performed on established RCC cell lines, whereas more relevant primary RCC cell cultures were not included. We have previously analyzed the adenoviral transduction of several established RCC cell lines and primary cell cultures with recombinant serotype 5 adenoviruses expressing the green fluorescent protein marker gene, Ad5-GFP. Almost complete transduction of six RCC cell lines (SKRC-1, SKRC-7, SKRC-10, SKRC-17, SKRC-59, and CaKi-1) was achieved at MOI levels between 1 and 10 (Fig. 1A), although a dichotomy was apparent. Moreover, highly efficient transduction at MOI = 1 was seen with four RCC primary cell cultures (EUNRC-AB, EUNRC-AR, EUNRC-8781, and EUNRC-12175) established at our institute, immediately after RCC tumor nephrectomy (Fig. 1B). The efficient transduction of the RCC primary cell cultures examined indicates relatively high levels of CAR expression, which was confirmed by flow cytometric analysis of CAR (Fig. 2). In contrast, the transduction rate of dendritic cells was found to be significantly lower with ≤10% of cells transduced at MOI ≤ 10 and >90% of the cells transduced at MOI ≥ 100 (data not shown). This inefficient transduction confirmed the observation of Rea et al. (2) showing that dendritic cells do not express CAR.

Although all primary RCC cell cultures displayed high transduction rates, the RCC cell lines can be divided into high and intermediate adenovirus transducible cells (Fig. 1A). For instance, the RCC cell line SKRC-17 belongs to the group of cells with a high transduction rate, whereas the RCC cell line CaKi-1 belongs to the group of cells with an intermediate transduction rate. These differences correlate with the membranous CAR expression on these cells (Fig. 2) and confirm the low CAR expression levels on the CaKi-1 cells as found by Haviv et al. (1).

To investigate whether the transduction efficiency of the RCC cell lines and primary cell cultures could be enhanced by targeting to the subgroup B adenovirus receptor, transduction by subgroup B (adenovirus serotype 35) fiber-modified serotype 5 adenovirus Ad5F35-GFP was studied. Such adenoviruses were found to be more efficient than conventional Ad5-GFP in transducing the CAR-negative dendritic cells (data not shown). Similarly, the RCC cell lines (SKRC-1, SKRC-10, and CaKi-1), which displayed intermediate transduction rates with Ad5-GFP, were more efficiently transduced with Ad5F35-GFP (Fig. 3A). In contrast, the RCC primary cell cultures (EUNRC-AB, EUNRC-AR, and EUNRC-8781) were equally susceptible for transduction by the Ad5F35-EGFP (Fig. 3B). In RCC cell lines with high transduction rates, no increase was observed (data not shown).

In conclusion, high to intermediate transduction rates of established RCC cell lines with serotype 5 adenovirus can be obtained. Although the intermediate transduction rates of the RCC cell lines with serotype 5 adenovirus can be improved with subgroup B fiber-modified serotype 5 adenovirus, this is not true for the RCC cell lines that already display high transduction rates with serotype 5 adenovirus. More relevant, the RCC primary cell cultures (passage number ≤ 3) examined displayed high transduction rates with serotype 5 adenovirus that could not be further improved with subgroup B fiber-modified serotype 5 adenovirus. Similar high transduction rates with serotype 5 adenovirus were seen with primary normal kidney cell cultures (data not shown). Although the number of cases examined in our study is limited, it indicates that CAR deficiency in primary RCC is not frequent. The CAR deficiency observed with some of the RCC cell lines, as also described by Haviv et al. (1), might be the consequence of the establishment of RCC cell line. Thus, for adenoviral gene therapy of renal cancer, retargeting to alternative cellular receptors is not required. However, the equal susceptibility for transduction of RCC cells with adenovirus serotypes 5 and 35 is an interesting finding when repeated adenoviral therapies would be considered. Then adenovirus serotype switching could be a promising approach in cancer gene therapy of RCC to overcome adenovirus serotype 5 neutralizing immune responses.

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References
Fig. 1. Transduction of RCC cells with Ad5-GFP recombinant adenovirus expressing the enhanced green fluorescent protein. Cells were infected at different MOIs, and GFP expression was determined 24 h postinfection by flow cytometry. A, RCC cell lines: SKRC-1, ▲; SKRC-7, ▼; SKRC-10, ●; SKRC-17, ◇; SKRC-59, ◆; and CaKi-1, ■. B, RCC primary cell cultures: EUNRC-AB, ▲; EUNRC-AR, ●; EUNRC-8781, ◇; and EUNRC-12175, ◆. Values shown are mean ± SD of multiple assays.

Fig. 2. CAR expression in RCC cells. Membranous CAR expression on cells of RRC cell lines (SKRC-17 and CaKi-1), RCC primary cell cultures (EUNRC-AB and EUNRC-8781), and human embryonic kidney control cell line 293 was determined by flow cytometry after staining with mouse antihuman CAR monoclonal antibody RmcB (bold lines) or an irrelevant isotype-matched antibody (dotted lines) followed by a secondary FITC-labeled goat antimouse antibody.

Fig. 3. Transduction of RCC cells with Ad5F35-GFP (open symbols) and Ad5-GFP (closed symbols) recombinant adenoviruses expressing the enhanced green fluorescent protein. Cells were infected at different MOIs, and GFP expression was determined 24 h postinfection by flow cytometry. A, RCC cell lines (low CAR): SKRC-1, triangle; SKRC-7, circle; SKRC-10, ▼; SKRC-17, ◇; and CaKi-1, square. B, RCC primary cell cultures: EUNRC-AB, ▲; EUNRC-AR, ◆; and EUNRC-8781, □. Values shown are mean ± SD of multiple assays.

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