Analysis of genetic heterogeneity in the HCAR adenovirus-binding Ig1 domain in a Caucasian Flemish population

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Abstract

Background: Polymorphisms in the gene that encodes the human cellular receptor for group B coxsackieviruses and adenoviruses (HCAR) could be responsible for differences in susceptibility to infections with these pathogens. Moreover, adenovirus subgroup C-mediated gene therapy could be influenced by mutations in the coding exons for the aminoterminal immunoglobulin-like 1 (Ig1) domain, which is the essential component for adenovirus fiber knob binding.

Results: Using two primersets in the adjacent intron sequences, HCAR exons 2 and 3, which comprise the full-length Ig1 domain, were amplified by polymerase chain reactions in 108 unselected and unrelated healthy Belgian volunteers. After nucleotide sequencing, no polymorphisms could be demonstrated in the adenovirus-binding Ig1 exons 2 and 3 of the HCAR gene.

Conclusions: The adenovirus-binding Ig1 domain seems to be a highly conserved region in the Caucasian population which is a reassuring finding regarding adenovector-based gene therapy.

Background

Recombinant human subgroup C adenoviruses (serotypes 2 and 5) are envisaged as efficient vector delivery systems in gene therapy because of their ability to transfect a wide variety of cells [1]. Successful gene delivery requires viral entry into the target cell via specific receptor-mediated uptake [2]. For adenoviruses from subgroups A, C, D, E and F, the human coxsackie-adenovirus receptor (HCAR) protein functions as the primary high-affinity binding site for the knob domains of the adenoviral fibers, elongating from the viral capsid structure. Subsequent interactions between the viral penton base and cell surface $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins induce virus internalization into the target cells [3].
acid residues involved in attachment of group B coxsackieviruses to HCAR may reside in the Ig2 domain (exons 4 and 5) or in an overlap region between Ig1 and Ig2 [6,7]. In contrast to thorough knowledge about the structure of HCAR and the viral binding mechanisms, little is known about the cellular function of this protein. A first report recently described that the mouse homologue of human CAR, that shows more than 80% similarity with the human cDNA-sequence, may function naturally as a cell adhesion molecule in the developing mouse brain [8]. HCAR tissue distribution and expression levels are important parameters influencing the efficiency of adenovirus-based gene delivery. Different groups reported a positive correlation between tissue HCAR levels and adenoviral infectivity [1,2,9]. In vivo, the receptor seems to be expressed preferentially in epithelial cells of multiple organs. The highest HCAR-mRNA expression was noted in heart, pancreas and brain whereas placenta and skeletal muscle were HCAR-negative [10].

Fundamental polymorphisms in the coding exons for the viral binding Ig1 and Ig2 domains, could be responsible for a variable susceptibility to infections with the respective pathogens and replication-deficient recombinant adenovectors. HCAR exons 2 and 3, which comprise the Ig1 domain, were therefore screened for mutations in 108 unrelated healthy Belgian individuals.

Results and Discussion

HCAR exons 2 and 3 were PCR-amplified in order to search for polymorphisms in the adenovirus-binding Ig1 domain. The exon 2 PCR generated an amplicon of 306 bp in length (exon 2 coding region: 167 bp), while a 339 bp fragment was amplified in the exon 3 PCR (exon 3 coding region: 205 bp). The resulting chromatograms were analyzed using the SeqMan multiple sequence alignment tool (LaserGene, DNAStar, Madison, WI). Consensus sequences were compared with the corresponding HCAR-sequences in Genbank using BLAST (Basic Local Alignment Search Tool) [12]. All the obtained sequences showed to be 100% identical to the sequence in Genbank (AF200465).

A previous report documented several key residues in the HCAR Ig1 domain that play an important role in the formation of a high-affinity adenovirus knob-HCAR complex [6]. Remarkable is that the sixteen predicted interfacial amino acids are wholly conserved among five different species, as we could deduce from the different CAR-sequences in Genbank (human: Y07593; mouse: Y10320; rat: AF109644; pig:AF109646; dog: AF109645). Mutational analysis of the Ig1 domain of HCAR demonstrated that single or multiple substitutions of these interfacial Ig1 residues could eliminate adenovirus attachment [6,7]. Polymorphisms in other regions of the HCAR-molecule might also indirectly affect adenoviral binding. Nevertheless, the Ig1 domain still remains the most important domain for adenovirus entry which has also been demonstrated by Wang and Bergelson [13] who stated that HCAR cytoplasmic and transmembrane domains are not essential for virus infection.

No polymorphisms could be revealed in exons 2 and 3 of the HCAR-gene among all 108 tested Caucasian individuals. The Ig1 domain that makes contact with the adenovirus fiber knob seems to be a highly conserved area in the HCAR protein, reinforcing the feasibility of adenovector-based gene therapy for a Caucasian population. Further research on other populations might be interesting.

Materials and Methods

DNA-samples of 108 unselected and unrelated Caucasian Flemish volunteers were collected through a non-invasive "swish-and-split" technique, by rinsing the oral cavity with 0.9% saline solution, after which genomic DNA was extracted using a simple alkaline lysis procedure [11]. Informed consent was obtained from all participants.

HCAR exons 2 and 3 (Genbank accession number AF200465) were amplified separately using polymerase chain reactions (PCR) with primers chosen in the adjacent intron sequences (HCAR-2F: 5’-TCAATGTGCTGCTTCTGAATG-3’ and HCAR-2R: 5’-GATAGTTGCACAGACAGCTGC-3’; HCAR-3F: 5’-TGTCACAGCAGGTGTATCCAG-3’ and HCAR-3R: 5’-CTCITACTGTAACCCGTAAAC-3’). (Figure 1). Samples were amplified in a final reaction volume of 50 µl, containing 0.1 µM forward and reverse primers, 0.5 mM dNTP’s, 2.5 mM MgCl₂, and 1 U Taq polymerase (Perkin Elmer/Roche Molecular Systems, Brussels, Belgium), pH 8.5. Both amplification profiles involved an initial denaturation at 94°C for 5 min, followed by a three step cycle of 30 sec at 94°C, 30 sec at 56°C (exon 2) or 53°C (exon 3) and 45 sec at 72°C for 35 cycles, and ending with a final extension of 5 min at 72°C in a GeneAmp PCR System 9700 thermal cycler (Perkin Elmer, Foster City, CA). PCR-products were run on a polyacrylamide gel, stained with ethidium bromide and visualized under UV-light. The amplification products were purified using the QIAquick PCR Purification Kit (Qiagen, Westburg, The Netherlands) and sequenced in forward and reverse
direction using the ABI PRISM BigDye Terminator Cycle Sequencing Reaction Kit on a ABI PRISM 310 DNA sequencer (Perkin Elmer Applied Biosystems, Foster City, CA), according to the manufacturer's instructions.

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