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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].

The gene that encodes HCAR is located on chromosome 21q11.2 and consists of seven exons that are distributed over an area of 54 kb [4]. After translation a 365-amino acid (aa) integral membrane glycoprotein is produced, with an N-terminal exoplasmic domain (218 aa), a single hydrophobic transmembrane-spanning region (21 aa) and a highly conserved cytoplasmic tail (107 aa) [5]. The extracellular portion of the receptor consists of two immunoglobulin-like domains: the N-terminal Ig1 is related to the immunoglobulin V fold and the more C-terminal Ig2 is related to the IgC2 fold. Structural analysis of the mechanism of adenovirus binding to HCAR revealed that only the Ig1 domain (exons 2 and 3) makes contact with the fiber knob. In contrast, molecular interactions of amino

Figure 1
Position of the forward and reverse primers in intron 1,2 and 3 of the HCAR gene.

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-spit" 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'-TGTAGCAGCAGGTGTATCCAG-3' and HCAR-3R: 5'-CTCTTACTCAGTAACCCGTTAACAC-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 $MgCl_2$, 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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References

1. Li Y, Pong R-C, Bergelson JM, Hall MC, Sagalowsky AI, Tseng C-P, Wang Z, Hsieh J-T: **Loss of adenoviral receptor expression in human bladder cancer cells: a potential impact on the efficacy of gene therapy.** *Cancer Res* 1999, **59**:325-330
2. Fechner H, Wang X, Wang H, Jansen A, Pauschinger M, Scherubl H, Bergelson JM, Schultheiss H-P, Poller W: **Trans-complementation of vector replication versus Coxsackie-adenovirus-receptor overexpression to improve transgene expression in poorly permissive cancer cells.** *Gene Ther* 2000, **7**:1954-1968
3. Bergelson JM: **Receptors mediating adenovirus attachment and internalization.** *Biochem Pharmacol* 1999, **57**:975-979
4. Bowles KR, Gibson J, Wu J, Shaffer LG, Towbin JA, Bowles NE: **Genomic organization and chromosomal localization of the human Coxsackievirus B-adenovirus receptor gene.** *Hum Genet* 1999, **105**:354-359
5. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW: **Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5.** *Science* 1997, **275**:1320-1323
6. Bewley MC, Springer K, Zhang Y-B, Freimuth P, Flanagan JM: **Structural analysis of the mechanism of adenovirus binding to its human cellular receptor, CAR.** *Science* 1999, **286**:1579-1583
7. Tomko RP, Johansson CB, Totrov M, Abagyan R, Frisen J, Philipson L: **Expression of the adenovirus receptor and its interaction with the fiber knob.** *Exp Cell Res* 2000, **255**:47-55
8. Honda T, Saitoh H, Masuko M, Katagiri-Abe T, Tominaga K, Kozakai I, Kobayashi K, Kumanishi T, Watanabe YG, Odani S, Kuwano R: **The coxsackievirus-adenovirus receptor protein as a cell adhesion molecule in the developing mouse brain.** *Mol Brain Res* 2000, **77**:19-28
9. Van't Hof W, Crystal RG: **Manipulation of the cytoplasmic and transmembrane domains alters cell surface levels of the coxsackie-adenovirus receptor and changes the efficiency of adenovirus infection.** *Hum Gene Ther* 2001, **12**:25-34
10. Fechner H, Haack A, Wang H, Wang X, Eizema K, Pauschinger M, Schoemaker RG, Van Veghel R, Houtsmuller AB, Schultheiss H-P, Lamers JMJ, Poller W: **Expression of Coxsackie adenovirus receptor and alpha$_v$-integrin does not correlate with adenovector targeting in vivo indicating anatomical vector barriers.** *Gene Ther* 1999, **6**:1520-1535
11. Lench N, Stanier P, Williamson R: **Simple non-invasive method to obtain DNA for gene analysis.** *Lancet* 1988, **1**:1356-1358
12. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: **Basic local alignment search tool.** *J Mol Biol* 1990, **215**:403-410
13. Wang X, Bergelson JM: **Coxsackievirus and adenovirus receptor cytoplasmic and transmembrane domains are not essential for coxsackievirus and adenovirus infection.** *J. Virol.* 1999, **73**:2559-2562