Generation of Recombinant Adenovirus Vectors Carrying Wild-type and Mutant MMP1 Genes

Adenovirus vectors carrying glucocorticoid inducible, full coding wild-type and mutant MMP1 cDNAs (AdhGRE.MMP1 and AdhGRE.mutMMP1) were generated by homologous recombination using the AdEasy Adenoviral Vector System (Stratagene, La Jolla, CA). For the wild-type, the MMP1 cDNA was obtained from RNA extracted from primary HTM cells overexpressing myocilin, which has been shown to increase expression of MMP1 by 26-fold. For the wild-type, the MMP1 cDNA was obtained from RNA extracted from primary HTM cells overexpressing myocilin, which has been shown to increase expression of MMP1 by 26-fold. Primary HTM-95 cells were infected with AdhTIG at a multiplicity of infection (moi) of 2.6 x 10^4 virus genomes/cell (vg/cell), RNA extracted at 72 hours post-infection and RT performed as indicated above. One μl of the RT reaction was amplified using high fidelity Advantage HD polymerase (Clontech, Mountain View, CA) (94°C 1 minute; 35 cycles: 98°C 10 seconds, 55°C 15 seconds, 72°C 100 seconds; 72°C 7 minutes), and primers 5'-AAGCTTCCACCATGCACAGCTTTCCTCCACTG-3' (forward) and 5'-GGCCGGCCTCAATTTTTCCTGCAGTTGA-3' (reverse). These primers were designed to contain HindIII and FseI sites at their 5' ends and a CCACC Kozak consensus sequence prior to the MMP1 ATG codon. The amplified 1,424 bp DNA fragment was gel purified and cloned into the pCR-blunt II-TOPO plasmid (Invitrogen) (pMG10) for sequence confirmation. For the MMP1 mutant (mutMMP1), the coding sequence was obtained by PCR amplification of plasmid #516 from our HTM1 library using the same primers, conditions and vector used to amplify and clone wild-type MMP1 (pMG1). Upon sequencing, pMG1 cDNA contained two point mutations at positions 653 and 1115 (position 1 is the A in the ATG initiation of translation codon).

Wild-type (pMG10) and mutant (pMG1) cloning plasmids were then digested with HindIII-FseI, purified, and cloned into a HindIII-FseI predigested pGRE-Luc vector (Clontech) immediately downstream of the transcription blocker (TrBlk), glucocorticoid regulatory element (GRE) and the TATA-like promoter (P_TAL) (pMG12 and pMG13, respectively). To generate recombinant adenoviruses, the MMP1 full expression cassettes (TrBlk.GRE.P_TAL.MMP1.pA and TrBlk.GRE.P_TAL.mutMMP1.pA) were NotI/SalI digested from vectors pMG12 and pMG13 and inserted at the same restriction sites into the promoterless pShuttle vector (Stratagene) (pMG17 and pMG18). These new vectors were linearized with Pmel and electroporated into BJ5183-Ad1 cells for the recombination with the adenovirus backbone plasmid (pAdEasy1) according to manufacturer’s directions. The resultant vectors (pMG19 and pMG20) were amplified in E. coli competent cells XL10-gold (Stratagene),
purified, linearized with PacI and transfected into early-passage QBI-HEK 293A (Qbiogene, Montreal, Canada) for the production of the recombinants (AdhGRE.MMP1 and AdhGRE.mutMMP1). High-titer viral stocks were obtained by propagation in the same cells and purification by double banding CsCl density centrifugation as previously described. The collected viral CsCl band was desalted with NAP-5 columns (GE Healthcare, Piscataway, NJ) equilibrated with virus vehicle (0.01 M Tris pH 7.4, 1 mM MgCl₂, 10% glycerol), aliquoted and saved at -80°C.