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.[31] Primary HTM-95 cells were infected with AdhTIG3[31] 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[38] 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 *PmeI* 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.[20] 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 $MgCl_2$, 10% glycerol), aliquoted and saved at -80°C.