"Supplement Material"

Materials and Methods

Plasmids: Sequences 1483 bp upstream and 83 bp downstream from the transcription initiation site of human *MTTP* gene were cloned into pGL2-Basic (Promega) between Hind III and Kpn I sites to generate pMTP-1483. A similar strategy was used to clone pMTP-1181 and pMTP-204. For pMTP-775, a Hind III-Bgl II DNA fragment was excised from pMTP-1483 and inserted into pGL2-Basic. All insert sequences were identical to the human genomic sequence AC083902. QuickChange site-directed mutagenesis (Stratagene) was performed in pMTP-204 to yield SREm, HNF1m, HNF4m, DR1m, and 2mut constructs harboring specific mutations in different *cis* elements. The primers (Table 1) used for cloning were designed using Primer 3.0. Plasmid expressing NrF1 (pMT2-NR2F1) was kindly provided by Dr. Sotirios Karanthanasis.

Cell cultures: For differentiation, approximately 3 x 10^5 Caco-2 cells were placed in Transwells (Corning) and cultured in DMEM supplemented with 20% FBS [1]. The media were changed every other day until day 16. Conditioned media and cell cultures were collected and kept at -70^{o}C until analysis. ApoB was quantified using ELISA [2, 3].

Infection of cells with adenoviruses: Caco-2 cells seeded at 30% confluence in 6-well plates. The next morning cells were washed once with DMEM containing 1%FBS and indicated MOI of adenovirus was added in 300 μl of DMEM containing 1%FBS. After 6 h incubation, 2 ml of DMEM supplemented with 10% FBS DMEM was added to each well and cells were allowed to grow overnight. Next day these infected Caco-2 cells were used for metabolic labeling or harvested for western blotting.

Transfection with plasmid DNA or siRNA: Cells in 12-well plates were 70% to 80% confluent on the day of transfection. Exgen 500 reagent (Fermentas) was used to deliver 500 ng of pMTP luciferase constructs with 20 ng of pCMV-RL (Promega) that expresses Renilla luciferase under the control of a cytomegalovirus promoter. After 48 h, cells were harvested for analyses. Variations in transfections were normalized to the expression of

Renilla luciferase. To stably transfect Caco-2 cells, we used 50 ng of pTRE2hyg (BD Bioscience) instead of pCMV-RL during transfection. After 2 days, cells were re-plated in 100-mm dishes and cultured in the presence of 300 μg/mL hygromycin. Colonies resistant to hygromycin were harvested after 2 weeks. Polyclonal cell lines were maintained in 100 μg/mL hygromycin. Lipofectamine 2000 reagent (Invitrogen) was employed to transfect 50 nM siRNA (Table 1) into Caco-2 cells at a ratio of 3.3 μl to 5 pmol siRNA. After overnight incubation, fresh media were added to dilute siRNA to 20 nM. Cells were collected for analysis after 2 days.

Immunofluorescent Staining: The cell staining procedure was described previously [4]. Tissue samples from C57BL/6J mice were fixed in 4% paraformaldehyde, frozen in Tissue-Tek OCT compounds (Fisher Scientific), sectioned onto Tissue Tack slides (Polyscience), and incubated with 0.05% Tween 20 in 10 mM sodium citrate buffer (pH 6.0) at 90^{o}C for 25 min. Slides were incubated in a blocking buffer, PBS containing 1% horse serum, 3% BSA and 1% Triton X-100, for 30 min before 1 hr incubation with primary antibodies followed by another hour incubation with secondary antibodies. All antibodies were diluted 1 to 300 in the blocking buffer and the whole immunostaining was carried out at room temperature [4], Images were obtained with a laser-scanning microscope (Model LSM 510, Carl Zeiss) and processed with Photoshop 6.0 before exporting to Illustrator CS2 (Adobe).

Separation of mouse intestinal epithelial cells: To isolate various intestinal cell types [5], jejunum (about 16 cm from the end of the duodenum) was removed from anesthetized mice, emptied, and rinsed with saline containing 1 mM dithiothreitol (DTT). The lumen was filled with 3 mL of 1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4 buffer pH 7.3, and incubated with continuous agitation in a 37^{o}C saline bath for 15 minutes. The lumen was emptied and refilled with 3 mL of 1 mM EDTA and 0.5 mM DTT in PBS and incubated in the saline bath for 4, 2, 2, 3, 4, 5, 7, 10 or 15 minutes to collect 9 fractions. Rinsing the lumen with 2 mL of saline between incubations increased the yield.

RNA preparation and quantitative real-time PCR (qRT-PCR): RNA from Caco-2 or mouse intestinal cells was used to synthesize cDNA. The quantitative PCR [6] was carried out with a SYBR Green I qPCR kit (Eurogenetic) using primers described in Table 1. Data were analyzed using the $\Delta\Delta C_T$ method and are presented as fold change.

Chromatin immunoprecipitation (ChIP): ChIP was used to study the binding of transcription factors to the *MTTP* promoter [7]. Four 100-mm Transwell dishes (Corning) of 4-day Caco-2 cultures or 2 dishes of 14-day cultures were fixed with 1% formaldehyde. Isolated primary enterocytes were fixed with 1.5% formaldehyde. After 15 minutes at room temperature, cells were harvested. Nuclear fractions were isolated and sonicated (Sonic Dismembrator 550, 15 sec at setting 5, 16 bursts, 10-sec intervals). Approximately 25 μg of cross-linked genomic DNA in 1 mL 1%BSA/PBS was incubated with 50 μL of antibodies against NR2F1 (sc-6577), HNF-4α (sc-8987) or HNF-1α (sc-10791) overnight at 4^{o}C. Next day 50 μL of protein A/G beads (sc-2003) were added and centrifuged at 8,000 rpm for 5 min at room temperature. Precipitated DNA was extracted by phenol-chloroform after reversal of cross-linkage by incubating at 65^{o}C for 4 h. Real-time and regular PCR were carried out using primers (Supplementary Table 1) for *MTTP*, *HNF4A*, or *APOB* promoters to determine the binding of different transcription factors. For controls, non-immune serum (sc-2028) was used.

Supplementary Table 1. Oligonucleotide sequences of primers and siRNAs

Primer pairs used for real-time RT-PCR		
Gene ID	**5' primer**	**3' primer**
NR2F1	GCGGTTCAGCGAGGAAGA	TGCGTACTGGCCTGGATTG
NR2F2	ATCGCAACCAGTGCCAGTACT	ACCGCTTCCCGTCTCATG
NR2F6	ACCGGAACCAGTGCCAGTAC	TGCACCGCCTCCTTCCT
Primer pairs used in ChIP experiments		
Promoter ID	**5' primer**	**3' primer**
APOB	GAAGCCAGTGTAGAAAAGCAAACA	CAAGAGCGCCAGGATTGG
MTTP[1]	CAGCCCACCTACGTTTAATCATT	CCAAACTCCAAAATCAGGACCTA
MTTP[2]	TGACCAGCAATCCTCAACTG	CCAAACTCCAAAATCAGGACCTA
HNF4A[3]	GGGTTCCCTAAGTGACTGGTT	ACCGCCCGGTTATCTTATTG
mttp	TAGCCCACCTGCGTTTAATCATT	CCAAACTCCAAAATCAGGACCTA
Primers for cloning of WT or mutant *MTTP* promoter		
Primer ID		
(*Hind III*) +83	*CCCAAGCTT*TGACCAGCAATCCTCAACTG	
(Bgl II) -204	*GGAAGATCT*GGTTTAGCTCTCAAAAGTGAAA	
(Kpn I) -1181	*CGGGGTACC*GGGTTTTGAGAGTGACCAG	
(*Kpn I*)-1483	*CGGGGTACC*CAAAGCACTTTCACATTGC	
Mut-SRE	GAGTGAGAGACTGAAAACTGC*gaattc*CCTACGTTTAATC	
Mut-HNF1	CCCACCTACGTT*g*AATCAT*g*AATAGTGAGCCC	
Mut-HNF4	GGTCCTGATTTTGGAGT*gc*GG*ccg*CTGACCTTTCCCCAAAG	
Mut-DR1	GGAGTTTGGAGTCTGA*tg*TTTCCCCAAAG	
Sequences of siRNA forward strands		
Gene ID	**Target sequences**	
NR2F1	rCrCrGrCrGrAUrAUrGUUrArCUrGUrCUTT	
NR2F2	rCrGrArGrArArCrAUUUrGrCrGrArArCUrGUU	
NR2F6	UrGrArCUrCrArArGrGrCrCrArAUrArAUrAUU	
GL2-luciferase	rCrGUrArCrGrCrGrGrArAUrArCUUrCrGrATT	

1. Primers for real-time PCR.
2. Primers for regular PCR.
3. β cell-specific P2 promoter, which is not accessible in intestinal cells.

Reference List

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(3) Bakillah A, Zhou Z, Luchoomun J, Hussain MM. Measurement of apolipoprotein B in various cell lines: correlation between intracellular levels and rates of secretion. *Lipids* 1997;32:1113-8.

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(6) Iqbal J, Dai K, Seimon T, Jungreis R, Oyadomari M, Kuriakose G, Ron D, Tabas I, Hussain MM. IRE1β inhibits chylomicron production by selectively degrading MTP mRNA. *Cell Metab* 2008;7:445-55.

(7) Sheng Z, Liang Y, Lin CY, Comai L, Chirico WJ. Direct regulation of rRNA transcription by fibroblast growth factor 2. *Mol Cell Biol* 2005 November;25:9419-26.