Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2014 September ; 34(9): 1917–1923. doi:10.1161/ATVBAHA.113.302806.

TMEM55B is a Novel Regulator of Cellular Cholesterol Metabolism

Marisa W. Medina, PhD[1], **Frederick Bauzon, PhD**[1], **Devesh Naidoo**[1], **Elizabeth Theusch, PhD**[1], **Kristen Stevens**[1], **Jessica Schilde, PhD**[2], **Christian Schubert, PhD**[5], **Lara M. Mangravite, PhD**[3], **Lawrence L. Rudel, PhD**[4], **Ryan E. Temel, PhD**[4], **Heiko Runz, MD**[2,5], and **Ronald M. Krauss, MD**[1]

[1]Children's Hospital Oakland Research Institute, Oakland, CA 94609

[2]Institute of Human Genetics, INF 366, 69120 Heidelberg, Germany

[3]Sage Bionetworks, Seattle, WA 98109

[4]Department of Pathology – Lipid Sciences, Wake Forest University Health Sciences, Winston-Salem, NC, USA

[5]Molecular Medicine Partnership Unit (MMPU), INF 350, 69120 Heidelberg, Germany

Abstract

Objective—Inter-individual variation in pathways impacting cellular cholesterol metabolism can influence levels of plasma cholesterol, a well-established risk factor for cardiovascular disease. Inherent variation among immortalized lymphoblastoid cell lines (LCLs) from different donors can be leveraged to discover novel genes that modulate cellular cholesterol metabolism. The objective of this study was to identify novel genes that regulate cholesterol metabolism by testing for evidence of correlated gene expression with cellular levels of 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) mRNA, a marker for cellular cholesterol homeostasis, in a large panel of LCLs.

Approach and Results—Expression array profiling was performed on 480 LCLs established from participants of the Cholesterol and Pharmacogenetics statin clinical trial, and transcripts were tested for evidence of correlated expression with *HMGCR* as a marker of intracellular cholesterol homeostasis. Of these, transmembrane protein 55b (*TMEM55B*) showed the strongest correlation (r=0.29, p=4.0E-08) of all genes not previously implicated in cholesterol metabolism and was found to be sterol regulated. *TMEM55B* knock-down in human hepatoma cell lines promoted the decay rate of the low density lipoprotein receptor (LDLR), reduced cell surface LDLR protein, impaired LDL uptake, and reduced intracellular cholesterol.

Conclusions—Here we report identification of *TMEM55B* as a novel regulator of cellular cholesterol metabolism through the combination of gene expression profiling and functional

Correspondence To: Marisa W. Medina, 5700 Martin Luther King Jr. Way, Oakland, CA 94609, Phone: 510-45-7977, Fax: 510-450-7909, mwmedina@chori.org.

Disclosures: none

studies. The findings highlight the value of an integrated genomic approach for identifying genes that influence cholesterol homeostasis.

Keywords

cholesterol; LDLR; post-transcriptional regulation; cardiovascular disease; PIP2

Introduction

Immortalized lymphoblastoid cell lines (LCLs), created by Epstein-Barr virus transformation of peripheral blood mononuclear cells[1], have been used as a model system to study genetic variation affecting cholesterol metabolism and statin response. LCLs were first used within the context of familial hypercholesterolemia (FH) to functionally assess the effects of specific FH mutations on LDLR cell surface protein and rates of LDL-uptake[2, 3]. In addition, we have previously performed cellular phenotyping of LCLs to identify the functional effects of genetic variation within 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) and the low density lipoprotein receptor (*LDLR*) associated with variation in plasma LDL-cholesterol both at baseline and in response to statin treatment[4-7].

More recently, studies of LCLs have moved beyond functional analysis of specific gene variants, and have proven to be informative for discovery-based purposes as well. For example, given the unclear genetic background of familial combined hyperlipidemia (FCHL), expression array analysis of LCLs from FCHL patients versus healthy controls was used to identify disease-specific transcriptomic profiles[8]. Through expression array analysis of 480 *in vitro* simvastatin and sham incubated LCLs derived from participants of the Cholesterol and Pharmacogenetics (CAP) statin clinical trial, we recently reported the identification of *RHOA* as a novel candidate gene implicated in LDL-cholesterol lowering in response to statin treatment[9]. Since *HMGCR* encodes the rate-limiting enzyme of the cholesterol biosynthesis pathway and is tightly regulated at the level of gene transcription by sterol response element binding factor 2 (SREBF2) in response to changes in intracellular sterol content[10], we identified genes whose statin-induced expression level changes were most highly correlated with statin-induced changes in *HMGCR*.

Here we sought to determine if we could extend that line of reasoning to cells in the untreated state to identify novel genes not previously implicated in cholesterol metabolism, hypothesizing that inter-individual variation in cellular *HMGCR* transcript levels could serve as a marker of genetic regulation of cellular cholesterol homeostasis. Using gene expression array data from a set of 480 LCLs from participants of the CAP clinical trial, we tested transcriptome-wide for genes with evidence of correlated expression with *HMGCR*. From this analysis transmembrane protein 55b (*TMEM55B*) emerged as the top novel candidate gene not previously implicated in cholesterol metabolism. Genetic manipulation of *TMEM55B* in hepatoma cell lines demonstrated that this gene is a novel regulator of cellular cholesterol metabolism that modulates LDLR levels and activity.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

I. Identification of TMEM55B as a candidate gene involved in cholesterol metabolism

To discover novel candidate genes involved in cellular cholesterol metabolism, we sought to identify genes whose expression levels were correlated with *HMGCR* transcript levels using genome-wide gene expression data from 480 LCLs derived from participants of the CAP clinical trial. From this analysis, 110 genes were identified to have correlated expression with *HMGCR*, Bonferroni adjusted $p<0.05$ (Tables 1, S2 for complete list). As expected, the genes most highly correlated with *HMGCR* transcript levels were those encoding enzymes within the cholesterol biosynthesis pathway as well as *LDLR*. From this list, transmembrane protein 55b (*TMEM55B*) was identified to be the gene most highly correlated ($r=0.29$, Bonferroni adjusted p-value 4.0E-08) with *HMGCR* transcript levels that has not been previously implicated in cholesterol metabolism.

II. TMEM55B is a novel sterol-regulated SREBF target gene

To test if endogenous *TMEM55B* transcript levels were changed in response to intracellular sterol content, we treated three human hepatoma cell lines, HepG2, Hep3B and Huh7, to conditions of extreme sterol depletion using media containing 2 μM simvastatin + 10% lipoprotein deficient serum (LPDS), thus reducing both cholesterol synthesis and uptake. After 24 hours, we added back sterols using either LDL-cholesterol or 25-hydroxycholesterol and subsequently quantified gene expression. *LDLR* gene expression, used as a positive control to verify incubation conditions, demonstrated the expected increase with sterol depletion and decrease with add-back. Similarly, extreme sterol depletion (statin + LPDS) induced *TMEM55B* transcript levels 1.7 to 2.8-fold depending on cell type, while sterol add-back reversed this effect (Figure 1A). Induction of *TMEM55B* transcript levels with statin treatment was also observed after *in vitro* simvastatin exposure (0.5 and 2.0μM) of primary human hepatocytes derived from four unrelated donors (Figure 1B). Since *LDLR* transcript level was not increased in a statistically significant manner across both statin concentrations, *HMGCR* transcript was quantified as a second positive control to validate the exposure conditions. Lastly, quantification of hepatic *TMEM55B* in African Green monkeys before and after a 19-week 0.6mg/kcal cholesterol supplemented diet found that cholesterol loading reduced *TMEM55B* transcript levels ∼40% (n=5 animals, $p=0.01$, Figure 1C). We observed similar results in an independent study of African Green monkeys fed a diet containing 0.002, 0.2 or 0.4 mg/kcal cholesterol for 10 weeks (n=5 per diet, Figure 1D), with cholesterol loading down-regulating*TMEM55B* transcript levels in a dose-dependent manner. Similar to results observed *in vitro*, cholesterol-induced changes in *TMEM55B* were of approximately the same magnitude as those observed in *LDLR* transcript levels. Furthermore, inter-individual variation in hepatic *TMEM55B* expression levels was directly correlated to variation in hepatic total cholesterol, free cholesterol and cholesterol esters (Figures 1E, SIA and SIB), whereas there was no relationship between variation in hepatic *LDLR* transcript levels with hepatic cholesterol ($r^2=0.01$ for total cholesterol). These results support the likelihood that inter-individual variation in hepatic cholesterol in response to cholesterol feeding is mediated in part by variation in *TMEM55B* expression levels.

Sterol regulation of *TMEM55B* transcript levels was not limited to liver-derived tissues since *TMEM55B* transcript levels were also up-regulated in simvastatin versus sham-treated CAP LCLs after 24 hours (1.2±0.01 fold, n=480, false discovery rate <0.0001). *TMEM55B* induction was also observed when sterol-depletion was performed by alternative means than statin treatment and in additional cell models. For instance, in HeLa cells and human fibroblasts cultured in lipoprotein-depleted serum and exposed to the cholesterol-reducing agent 2-hydroxyl-β-cyclodextrin, *TMEM55B* transcript was up-regulated by 2.4±0.7 fold (n=4, p=0.13) and 3.4±0.6 fold (n=3, p=0.05), respectively.

Transcript levels of genes involved in the maintenance of intracellular cholesterol levels are primarily regulated by the sterol-dependent transcription factors SREBF1 and SREBF2 (aka SREBP1 and 2)[10]. We queried publicly available SREBF2 ChIP-Seq datasets[11], and found evidence for SREBF2 binding motifs in the promoter region of the *TMEM55B* gene (chr14:20,929,629-20,930,186; GRCh37/hg19) in both HepG2 and a human immortalized lymphoblastoid cell line, Figure SIB. To confirm SREBF2-regulation of *TMEM55B* gene expression, we quantified *TMEM55B* in Huh7 cells transfected with siRNAs targeting SREBF1, SREBF2, both SREBF1 + SREBF2, or a non-targeting control (NTC). Both SREBF1 and 2 knock-down reduced *TMEM55B* transcript levels ∼30% (p=0.03 and p=0.04 respectively) with no additive effect of the dual knock-down (Figure SIC). Similar results were observed in HeLa cells, where SREBF2 knock-down suppressed sterol-dependent up-regulation of *TMEM55B* (Figure SID).

IV. TMEM55B modulates intracellular cholesterol levels and LDLR metabolism

We next sought to determine if TMEM55B modulates cellular cholesterol levels. We transfected human hepatoma cell lines with TMEM55B-siRNAs (aka TMEM-1 and TMEM-2) that reduced *TMEM55B* transcript levels and protein levels ∼80% and 60% respectively (Figures 2A and 2B, SII). Importantly, TMEM55B knock-down in HepG2 reduced overall cellular total cholesterol (0.64±0.1 fold, n=8, p=0.01), free cholesterol (0.70±0.07 fold, n=8, p=0.008) as well as cholesterol esters (0.39±0.1 fold, n=8, p=0.05), Figure 2C. Similar results were obtained with an independent TMEM55B-siRNA as well as in Huh7 cells. Despite the changes in cellular cholesterol levels, we did not find indications for differences in APOB, APOE or APOCIII in the conditioned media of TMEM55B knock-down HepG2 or Huh7 cells (Figure SIIIA).

Intracellular cholesterol levels are regulated in part by cholesterol uptake via the low density lipoprotein receptor (LDLR)[12]. Since TMEM55B has been shown to localize to the endosome/lysosome[13], and LDLR recycles between this compartment and the cell surface[12], we hypothesized that TMEM55B could modulate LDLR turnover, activity or localization. To determine the potential effect of TMEM55B on LDLR protein, we first transfected HepG2 cells with either siRNA targeting *TMEM55B* or a non-targeting control siRNA (NTC) for 48 hours, then treated the cells with cycloheximide, an inhibitor of protein synthesis, and finally collected samples over three hours. Although we did not observe detectable degradation of total cellular LDLR protein in the NTC treated cells, TMEM55B knock-down caused a reduction in LDLR protein over three hours, consistent with an accelerated LDLR protein decay rate (Figure 2D, SIIIB).

Next, we analyzed whether TMEM55B knockdown reduced cell surface LDLR protein. As shown in Figure 2E, cell surface LDLR was reduced in HepG2 cells transfected with either TMEM-1 (0.87±0.05 fold, n=12, p=0.02) or TMEM-2 (0.79±0.05 fold, n=12, p=0.001), as well as in a second hepatoma cell line, Huh7, transfected with TMEM-2 (0.75±0.08 fold, n=12, p=0.02). Consistent with the reduction in cell surface LDLR protein, we also found that TMEM55B knockdown reduced LDL uptake in HepG2 cells transfected with either TMEM-1 (0.92±0.02, n=20, p=0.004) or TMEM-2 (0.92±0.02, n=20, p=0.001), as well as in Hep3B cells transfected with either TMEM-1 (0.95±0.02, n=12, p=0.03) or TMEM-2 (0.94±0.02, n=12, p=0.01), Figure 2F. Overexpression of TMEM55B in HepG2 cells produced the opposite effects, with increases of both cell surface LDLR protein and DiI-LDL uptake relative to control transfected cells (Figures 2E and 2F). We found no evidence that TMEM55B knock-down influenced either levels of cell surface transferrin receptor or rate or transferrin-568 uptake (Figures SIIIC and SIIID). Lastly, we found that TMEM55B knockdown with either TMEM-1 or TMEM-2 in both HepG2 and Huh7 cells did not reduce *LDLR* transcript levels, but rather caused a 20-50% increase under all conditions tested, Figure SIV. Notably, TMEM55B knock-down did not generate consistent changes in transcript levels of other SREBF2 target genes such as *HMGCR* or *PCSK9* (Figure SIV). These results support the hypothesis that TMEM55B modulates LDLR cell surface protein levels through a post-transcriptional process.

Discussion

Here, we have used genome-wide gene expression levels results from LCLs to identify candidate loci associated with *HMGCR* transcript levels, which serve as a biomarker for variation in cellular cholesterol metabolism, from which *TMEM55B* emerged as a novel candidate gene associated with cellular cholesterol metabolism. Recently, we have successfully used a similar approach to identify *RHOA* as a novel determinant of LDL-cholesterol response to statin treatment[9]. Although cellular phenotyping of LCLs has long been employed to functionalize the molecular impact of genetic variation associated with elevated cholesterol levels[2-4, 6, 9], to our knowledge this is the first instance of capitalizing on the inter-individual variation of a cellular phenotype within LCLs to identify novel genes involved in cellular cholesterol homeostasis.

TMEM55B, also known as Type I phosphatidylinositol 4,5-bisphosphate 4-phosphatase, was first identified as an enzyme that catalyzes the hydrolysis of 4-position phosphate on phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P_2 aka PIP_2) but not other phosphatidylinositols, to generate phosphatidylinositol-5-phosphate (PtdIns-5-P or PI(5)P)[13]. Phosphatidylinositides (PIs) control the timing and localization of endocytic membrane trafficking by recruiting components of the transport machinery, thus regulating intracellular membrane traffic. It has been well established that PIP_2, the predominant PI formed at the plasma membrane, is required for clathrin-mediated endocytosis, with roles in clathrin-coated pit initiation, stabilization, and maturation[14-17]. Since one of the major mechanisms for cholesterol uptake is through clathrin-mediated endocytosis of LDL-bound LDLR, it is possible that TMEM55B modulates LDLR activity through the regulation of PIs.

In support of that hypothesis, here we show that TMEM55B knockdown stimulated LDLR protein decay, resulting in reduced cell surface LDLR protein and LDL uptake. The increased LDL uptake seen with TMEM55B overexpression was consistent with positive correlation noted between inter-individual variation in hepatic *TMEM55B* transcript levels and hepatic total cholesterol from two independent experiments of cholesterol-fed African green monkeys (greater *TMEM55B* transcript leads to greater LDLC uptake, which leads to greater hepatic cholesterol). Importantly, we did not observe a relationship between variation in *LDLR* transcript levels and hepatic cholesterol, suggesting that the relationship observed is not due to variation in sterol-induced changes in *TMEM55B* transcript levels. Thus, these results support the likelihood that variation in hepatic TMEM55B may directly modulate cholesterol metabolism.

TMEM55B may also modulate LDLR regulation through a non-clathrin mediated mechanism. For example, the F-actin network structure, which impedes LDLR trafficking by creation of a physical barrier to vesicle movement, has also been shown to be dependent on PIP_2 concentrations[18]. In addition, PI(5)P has been hypothesized to play a role in exocytosis from late endosome compartments and mediate TMEM55B effects on EGF-receptor decay rates[19, 20]. Since nascent LDLR is transported to the plasma membrane through the endoplasmic reticulum-Golgi pathway, and mature LDLR is known to recycle between the plasma membrane and endosome/lysosomes, it is possible that TMEM55B effects on PIs other than PIP_2 may impact LDLR intracellular trafficking. The importance of proper intracellular trafficking/signaling and sorting of LDLR has been recently shown by Zeigerer *et al* who reported that hepatic Rab5 knock-down in mice caused a ∼10-fold increase in plasma LDL levels compared to controls due to a reduction of the entire endolysosomal system (early and late endosomes, and lysosomes), increasing the number of clathrin-coated pits and vesicles and reducing the rate of LDL uptake[21]. Further studies are necessary to determine the precise mechanism by which TMEM55B regulates LDLR.

Notably there are over 40 kinases and phosphatases that impact PI metabolism, however *TMEM55B* appears to be the only gene in this pathway that is sterol-responsive (*personal communication ET, MWM, HR).* Furthermore, while TMEM55B might be expected to generally impact trafficking through the endolysosomal system, we did not observe an effect of TMEM55B knock-down on either levels of cell surface transferrin receptor or amount of 568-labeled transferrin within endosome-like particles, suggesting that LDLR is particularly sensitive to the effects of TMEM55B.

Although our studies strongly support the likelihood that TMEM55B directly modulates LDLR, interestingly we found that TMEM55B knock-down reduced intracellular cholesterol (total, free and esters) between 30-60%, while the reduction in LDLR cell surface protein and LDL uptake was closer to 10%. This discrepancy suggests that TMEM55B may modify other pathways that influence cellular cholesterol metabolism such as cholesterol efflux or synthesis. A key component of the insulin-signaling cascade is the activation of phosphoinositide 3-kinase (PI3-kinase) which phosphorylates PIP_2 to produce phosphatidylinositide (3,4,5)-triphosphate (aka PIP_3). Notably, VLDL synthesis and secretion is regulated by insulin. Cellular models have shown that generation of PIP_3 is necessary for insulin-mediated suppression of VLDL[22], while inhibition of PIP_3 generation

in vivo enhances hepatic VLDL APOB secretion[23, 24]. Since both TMEM55B and PI3-kinase share PIP_2 as a substrate, it is possible that TMEM55B knock-down impacts intracellular cholesterol levels through changes in VLDL metabolism. Similarly, activation of phosphatidylinositol-specific phospholipase C, an enzyme that also catalyzes the hydrolysis of PIP_2, has been previously linked to HDL-induced cholesterol efflux[25]. Thus, further studies are necessary to determine the precise mechanism(s) by which TMEM55B influences cholesterol metabolism.

In summary, using a novel approach of testing for evidence of correlated expression with *HMGCR*, we have identified *TMEM55B* as a novel gene that influences cholesterol homeostasis. We have validated this finding by functional studies demonstrating that TMEM55B regulates cellular levels of cholesterol and LDLR. Since increased LDLR activity is a major determinant of the clinical efficacy of statins and other drugs used for the prevention and treatment of cardiovascular disease, these results have implications for the development of novel drug targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

none

Sources of Funding: This work was supported by NIH NHLBI R01 HL104133, U19 HL069757, PO1-HL049373 (LLR), R00 HL088528 (RET), American Heart Association Fellowship 12POST10430005 as well as a Career Development Award (12CDA04) and the Transatlantic Networks of Excellence in Cardiovascular Research Program (10CVD03) of the Foundation Leducq.

References

1. Pressman S, Rotter JI. Epstein-barr virus transformation of cryopreserved lymphocytes: Prolonged experience with technique. Am J Hum Genet. 1991; 49:467. [PubMed: 1651051]
2. Tveten K, Ranheim T, Berge KE, Leren TP, Kulseth MA. Analysis of alternatively spliced isoforms of human LDL receptor mRNA. Clin Chim Acta. 2006; 373:151–157. [PubMed: 16828075]
3. Lombardi P, de Wit E, Frants RR, Havekes LM. Characterisation of the LDL receptor in epstein-barr virus transformed lymphocytes. Biochim Biophys Acta. 1990; 1044:127–132. [PubMed: 2160281]
4. Mangravite LM, Medina MW, Cui J, Pressman S, Smith JD, Rieder MJ, Guo X, Nickerson DA, Rotter JI, Krauss RM. Combined influence of LDLR and HMGCR sequence variation on lipid-lowering response to simvastatin. Arterioscler Thromb Vasc Biol. 2010; 30:1485–1492. [PubMed: 20413733]
5. Medina MW, Gao F, Naidoo D, Rudel LL, Temel RE, McDaniel AL, Marshall SM, Krauss RM. Coordinately regulated alternative splicing of genes involved in cholesterol biosynthesis and uptake. PLoS One. 2011; 6:e19420. [PubMed: 21559365]
6. Medina MW, Gao F, Ruan W, Rotter JI, Krauss RM. Alternative splicing of 3-hydroxy-3-methylglutaryl coenzyme a reductase is associated with plasma low-density lipoprotein cholesterol response to simvastatin. Circulation. 2008; 118:355–362. [PubMed: 18559695]
7. Gao F, Ihn HE, Medina MW, Krauss RM. A common polymorphism in the LDL receptor gene has multiple effects on LDL receptor function. Hum Mol Genet. 2013; 22:1424–1431. [PubMed: 23297366]

8. Morello F, de Bruin TW, Rotter JI, Pratt RE, van der Kallen CJ, Hladik GA, Dzau VJ, Liew CC, Chen YD. Differential gene expression of blood-derived cell lines in familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol. 2004; 24:2149–2154. [PubMed: 15388524]
9. Medina MW, Theusch E, Naidoo D, Bauzon F, Stevens K, Mangravite LM, Kuang YL, Krauss RM. RHOA is a modulator of the cholesterol-lowering effects of statin. PLoS genetics. 2012; 8:e1003058. [PubMed: 23166513]
10. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature. 1990; 343:425–430. [PubMed: 1967820]
11. Reed BD, Charos AE, Szekely AM, Weissman SM, Snyder M. Genome-wide occupancy of SREBP1 and its partners HFY and SP1 reveals novel functional roles and combinatorial regulation of distinct classes of genes. PLoS Genet. 2008; 4:e1000133. [PubMed: 18654640]
12. Goldstein JL, Brown MS. The LDL receptor. Arterioscler Thromb Vasc Biol. 2009; 29:431–438. [PubMed: 19299327]
13. Ungewickell A, Hugge C, Kisseleva M, Chang SC, Zou J, Feng Y, Galyov EE, Wilson M, Majerus PW. The identification and characterization of two phosphatidylinositol-4,5-bisphosphate 4-phosphatases. Proc Natl Acad Sci U S A. 2005; 102:18854–18859. [PubMed: 16365287]
14. Jost M, Simpson F, Kavran JM, Lemmon MA, Schmid SL. Phosphatidylinositol-4,5-bisphosphate is required for endocytic coated vesicle formation. Current biology:CB. 1998; 8:1399–1402. [PubMed: 9889104]
15. Zoncu R, Perera RM, Sebastian R, Nakatsu F, Chen H, Balla T, Ayala G, Toomre D, De Camilli PV. Loss of endocytic clathrin-coated pits upon acute depletion of phosphatidylinositol 4,5-bisphosphate. Proc Natl Acad Sci U S A. 2007; 104:3793–3798. [PubMed: 17360432]
16. Antonescu CN, Aguet F, Danuser G, Schmid SL. Phosphatidylinositol-(4,5)-bisphosphate regulates clathrin-coated pit initiation, stabilization, and size. Molecular biology of the cell. 2011; 22:2588–2600. [PubMed: 21613550]
17. Abe N, Inoue T, Galvez T, Klein L, Meyer T. Dissecting the role of ptdins(4,5)p2 in endocytosis and recycling of the transferrin receptor. J Cell Sci. 2008; 121:1488–1494. [PubMed: 18411250]
18. Aschenbrenner L, Naccache SN, Hasson T. Uncoated endocytic vesicles require the unconventional myosin, MYO6, for rapid transport through actin barriers. Molecular biology of the cell. 2004; 15:2253–2263. [PubMed: 15004223]
19. Lecompte O, Poch O, Laporte J. Ptdins5p regulation through evolution:Roles in membrane trafficking. Trends Biochem Sci. 2008; 33:453–460. [PubMed: 18774718]
20. Haucke V. Phosphoinositide regulation of clathrin-mediated endocytosis. Biochem Soc Trans. 2005; 33:1285–1289. [PubMed: 16246100]
21. Zeigerer A, Gilleron J, Bogorad RL, et al. Rab5 is necessary for the biogenesis of the endolysosomal system in vivo. Nature. 2012; 485:465–470. [PubMed: 22622570]
22. Qiu W, Federico L, Naples M, Avramoglu RK, Meshkani R, Zhang J, Tsai J, Hussain M, Dai K, Iqbal J, Kontos CD, Horie Y, Suzuki A, Adeli K. Phosphatase and tensin homolog (PTEN) regulates hepatic lipogenesis, microsomal triglyceride transfer protein, and the secretion of apolipoprotein B-containing lipoproteins. Hepatology. 2008; 48:1799–1809. [PubMed: 19026012]
23. Chirieac DV, Davidson NO, Sparks CE, Sparks JD. PI3-kinase activity modulates APO Bavailable for hepatic VLDL production in Apobec-1-/- mice. American journal of physiology. Gastrointestinal and liver physiology. 2006; 291:G382–388. [PubMed: 16798720]
24. Moon BC, Hernandez-Ono A, Stiles B, Wu H, Ginsberg HN. Apolipoprotein B secretion is regulated by hepatic triglyceride, and not insulin, in a model of increased hepatic insulin signaling. Arterioscler Thromb Vasc Biol. 2012; 32:236–246. [PubMed: 22155452]
25. Drobnik W, Mollers C, Resink T, Schmitz G. Activation of phosphatidylinositol-specific phospholipase C in response to HDL3 and LDL is markedly reduced in cultured fibroblasts from Tangier patients. Arterioscler Thromb Vasc Biol. 1995; 15:1369–1377. [PubMed: 7670951]

Abbreviations

LCLs	immortalized lymphoblastoid cell lines

HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase
TMEM55B	transmembrane protein 55b
LDLR	low density lipoprotein receptor
SREBF2	sterol response element binding factor 2
LPDS	lipoprotein-depleted serum
APO	apolipoprotein
NTC	non-targeting control
PIP_2	phosphatidylinositol-(4,5)-bisphosphate

Significance

Activity of the low-density lipoprotein receptor (LDLR) is a major determinant of circulating levels LDL cholesterol, a well-established risk factor for cardiovascular disease. Here we report identification of *TMEM55B* as a novel regulator of both LDLR and cellular cholesterol metabolism using a combination of gene expression profiling and functional studies. Nascent LDLR is transported to the plasma membrane through the endoplasmic reticulum-Golgi pathway, and mature LDLR is known to recycle between the plasma membrane and endosome/lysosomes. TMEM55B catalyze the conversion of phosphatidylinositides, or membrane bound signaling molecules that control the timing and localization of intracellular trafficking, suggesting that TMEM55B regulates LDLR by modulating its intracellular movement. Greater understanding of the molecular mechanisms guiding LDLR recycling may be useful for both improving our understanding of the pathways underlying inter-individual variation in LDL cholesterol, but also informing the development of novel drugs for the treatment and prevention of cardiovascular disease.

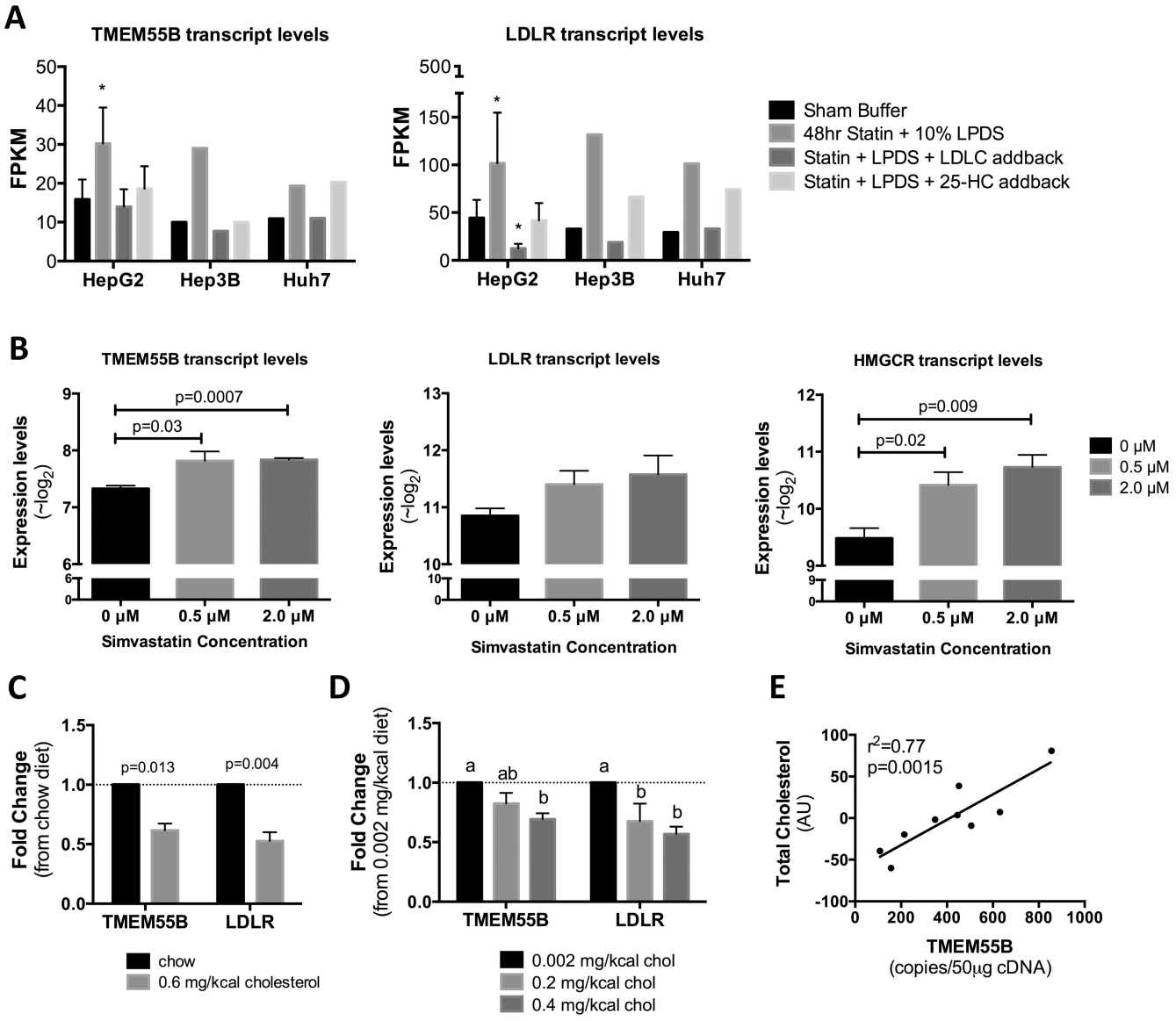

Figure 1. *TMEM55B* is sterol regulated

A. Three human hepatoma cell lines, HepG2 (n=10), Hep3B (n=1) and Huh7 (n=1) cells were exposed to one of four conditions 1) 48hr standard culture conditions, 2) 48hr 2µM simvastatin + 10% lipoprotein deficient serum (LPDS), 3) 24hr 2µM simvastatin + 10% LPDS, 24hr 2µM simvastatin + 10% LPDS + 50µg/ml LDLC, and 4) 24hr 2µM simvastatin + 10% LPDS, 24hr 2µM simvastatin + 10% LPDS + 1µg/ml 25-hydroxycholesterol. *TMEM55B* and *LDLR* transcript levels were quantified by RNA-seq. Values shown are fragments per kilobase of exon per million fragments (FPKM) mapped with 95% confidence intervals for HepG2 cells and FPKM values only for Hep3B and Huh7 cells. *Value is different from sham buffer alone, $p<0.05$. **B**. *TMEM55B*, *LDLR* and *HMGCR* transcript levels quantified in primary human hepatocytes from four unrelated donors after 24 hour *in vitro* incubation with 0, 0.5 and 2.0µM simvastatin. Values shown are variance stabilized fragment counts (which approximate a $\log_2$ transformation) corrected for library size

outputted from DESeq. Statistically significant differences were identified using two-tailed paired t-tests. **C.** Hepatic *TMEM55B* and *LDLR* transcript levels quantified in African green monkeys before and after a 19-week diet supplemented with 0.6mg/kcal cholesterol (n=5). **D.** Hepatic *TMEM55B* and *LDLR* transcript levels quantified in African green monkeys fed 0.002, 0.2 and 0.4 mg/kcal cholesterol for 10 weeks (n=5 animals per group). One-way ANOVA was used to identify statistically significant differences ($p<0.05$ for both *TMEM55B* and *LDLR* transcript levels) with a student's t-test performed post-hoc to identify differences between groups. **E.** Correlation between inter-individual variation in hepatic total cholesterol and hepatic *TMEM55B* transcript levels in animals on the 0.2 or 0.4 mg/kcal cholesterol supplemented diets. Cholesterol levels were adjusted for dietary cholesterol supplementation. Linear regression was performed in JMP 9.0.

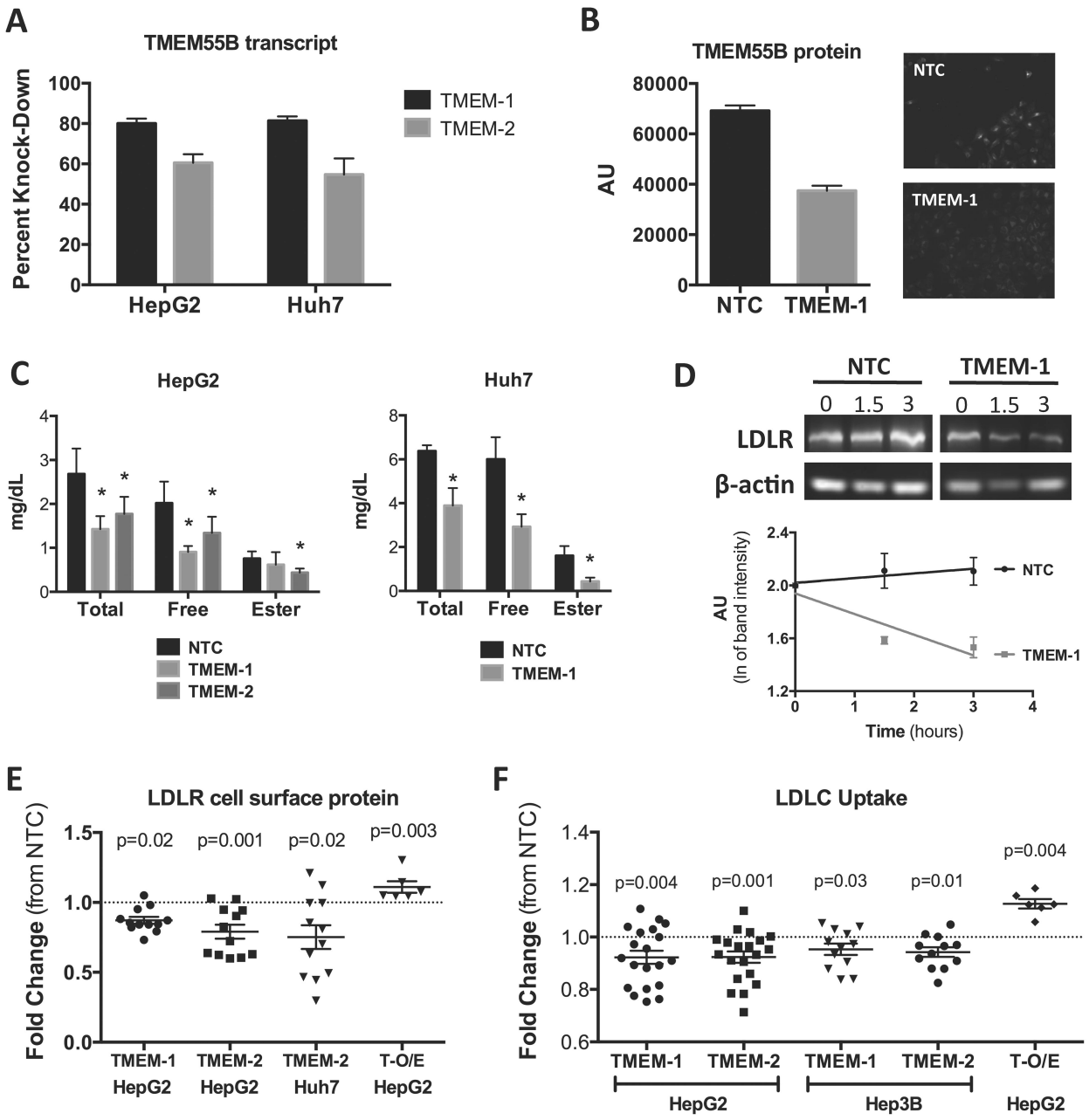

Figure 2. TMEM55B knock-down modulates LDLR

HepG2, Huh7 or Hep3B cells were transfected with siRNAs specific for TMEM55B (TMEM-1 or TMEM-2) or a non-targeting negative control (NTC) for 48hr. Where indicated, HepG2 cells were transfected with either an empty vector or a *TMEM55B* expression plasmid. **A.** *TMEM55B* transcript levels were quantified by qPCR (n=8 per cell line and siRNA). **B.** HeLa cells expressing *TMEM55B-GFP* together with either TMEM55BsiRNA or control siRNA were quantified for total cell-associated GFP-signal. Data was obtained from 300-400 cells from 10 background-subtracted images per condition (n=4 experiments). One set of representative images are shown. **C**. Intracellular cholesterol was quantified by the Amplex® Red Cholesterol Assay Kit in HepG2 (n=8) and Huh7 (n=6)

cells. **D.** HepG2 cells (n=3) were transfected with TMEM-1 or NTC siRNAs for 48 hours, after which cycloheximide was added to inhibit protein synthesis. Aliquots were collected over three hours, and total LDLR protein was quantified by western blot with values normalized to β-actin. Values shown are log transformed and expressed relative to time 0. **E.** LDLR protein was quantified after TMEM55B knock-down in HepG2 (n=12) and Huh7 cells (n=12), or TMEM55B overexpression (HepG2, n=6) via FACS as previously described[4]. **F**. LDL uptake was quantified in HepG2 cells (n=14) and Hep3B cells (n=12) after TMEM55B knock-down or in HepG2 cells after TMEM55B overexpression (n=6) using DiI-labeled LDL as previously described[4]. Paired two-tailed t-tests were used to calculate statistically significant differences in gene expression between NTC and TMEM55B siRNA transfected cells or pCMV (empty vector) and TMEM55B expression plasmid transfected cells.

Table 1

Genes whose transcript levels are correlated with *HMGCR* based on expression array data of 480 LCLs (r>0.25). A complete list of genes with evidence of correlation (Bonferroni adj p<0.05) can be found in Table SII.

Gene Symbol	Correlation Coefficient (r)	Adj P-value
HMGCS1	0.63	6.75E-51
SC4MOL	0.52	8.56E-32
SQLE	0.48	8.97E-26
LDLR	0.45	1.13E-22
TM7SF2	0.43	5.42E-20
MVK	0.41	1.49E-18
LSS	0.41	8.99E-18
NSDHL	0.40	1.99E-17
MVD	0.40	6.10E-17
SLC25A1	0.33	4.69E-11
IDI1	0.31	8.03E-10
TMEM97	0.30	1.07E-08
SCD	0.29	3.10E-08
TMEM55B*	0.29	4.00E-08
HSD17B7P2*	0.27	5.65E-07
LIPG	0.27	9.50E-07
IDH1*	0.26	2.44E-06
TDRD7*	0.26	3.13E-06
RHOB*	0.26	5.03E-06
TCTA*	0.26	5.98E-06

*Indicates a gene not previously implicated in lipid metabolism.