FEBS Letters 580 (2006) 5423–5428

# Modulation of LRP6-mediated Wnt signaling by molecular chaperone Mesd

Yonghe Li[a,b,*], Wenyan Lu[a,b], Xi He[c], Guojun Bu[a,d]

[a] Department of Pediatrics, Washington University School of Medicine and St. Louis Children's Hospital, St. Louis, MO 63110, USA
[b] Department of Biochemistry and Molecular Biology, Drug Discovery Division, Southern Research Institute, Birmingham, AL 35205, USA
[c] Division of Neuroscience, Children's Hospital, Department of Neurology, Harvard Medical School, Boston, MA 02115, USA
[d] Department of Cell Biology and Physiology, Washington University School of Medicine, MO 63110, USA

Received 3 May 2006; revised 1 September 2006; accepted 7 September 2006

Available online 18 September 2006

Edited by Lukas Huber

**Abstract** **LRP6 is a Wnt coreceptor at the cell surface. Here, we report that a specialized molecular chaperone Mesd modulates LRP6-mediated Wnt signaling and how different LRP6 mutants exhibit differential effects on Wnt signaling. We found that overexpression of increasing amounts of the full-length LRP6 enhances Wnt signaling in a dose dependent manner only in the presence of a co-expression of the molecular chaperone Mesd, which promotes LRP6 folding and maturation to the cell surface. We also demonstrated that LRP6 mutant lacking the intracellular domain impedes LRP6 cell surface expression and Wnt signaling in a dominant-negative fashion by sequestering Mesd from promoting LRP6 folding. Our results present novel mechanisms by which Mesd and LRP6 modulate Wnt signaling.**


*Keywords:* LRP6; Wnt signaling; Mesd; Chaperone

## 1. Introduction

The low-density lipoprotein receptor (LDLR)-related protein-5 (LRP5) and LRP6 are two members of the expanding LDLR family, which are a group of cell surface endocytosis receptors that are able to bind and internalize a wide array of extracellular ligands [1–3]. One of the predominant characteristics of the LDLR family members is the presence of multiple classes of conserved modules that include the β-propeller/epidermal growth factor (EGF) modules and clusters of cysteine-rich ligand-binding repeats. LRP5/6 has four β-propeller/EGF modules and three ligand-binding repeats [4–6]. All the currently characterized extracellular ligands of LRP5/6 bind to the β-propeller/EGF repeat modules [7–10], whereas ligands for other LDLR family members typically bind to the clusters of ligand-binding repeats [2,3].

A common feature that is shared by most members of the LDLR family is their ability to bind the receptor-associated protein (RAP) [11]. RAP is an endoplasmic reticulum (ER)-resident protein that functions in receptor folding and trafficking along the secretory pathway. Upon binding to receptors following their translation, RAP promotes receptor folding. In addition, RAP universally antagonizes ligand binding to these receptors [11]. Another chaperone that is specifically required for the biogenesis of the LDLR family members, termed Boca/Mesd, was recently described [12,13]. It was subsequently shown that Boca/Mesd promotes the proper folding of the β-propeller/EGF repeat modules of LRP5/6 [14]. More recently, we found that Mesd, like RAP, binds to cell surface LRP5/6 and antagonizes ligand binding [15].

The canonical Wnt signaling pathway is involved in various differentiation events during embryonic development and can lead to tumor formation when aberrantly activated [16,17]. Studies in the past several years have demonstrated that LRP5 and LRP6 are indispensable co-receptors of the canonical Wnt pathway by interacting with several key components of the Wnt/β-catenin signaling pathway [1,18]. Interestingly, it has been found that LRP5/6 mutant lacking the cytoplasmic tail is a dominant negative mutant for Wnt/β-catenin signaling [19–22], whereas LRP5/6 mutant without the extracellular domain is a constitutive activator [9,19,23,24]. These two types of LRP5/6 mutants are frequently used in Wnt signaling studies in many areas including embryonic development and tumorigenesis [5,9,19,21,22,24]. However, the mechanism by which LRP5/6 dominant negative mutants block Wnt signaling was unclear. In the present study, we further characterized Wnt signaling mediated by LRP6, and found that both the extracellular domain and the cytoplasmic tail of LRP6 are required for efficiently Wnt signaling, and that LRP6 mutant lacking the cytoplasmic tail could impede LRP6 expression and function through sequestering Mesd protein.

*Corresponding author. Address: Department of Biochemistry and Molecular Biology, Drug Discovery Division, Southern Research Institute, Birmingham, AL 35205, USA. Fax: +1 205 581 2093.
*E-mail address:* y.li@sri.org (Y. Li).

*Abbreviations:* EGF, epidermal growth factor; ER, endoplasmic reticulum; LDLR, low-density lipoprotein receptor; LRP6, LDLR-related protein-6; RAP, receptor-associated protein; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis

## 2. Materials and methods

### 2.1. Constructs

Plasmid pCS-Myc-hLRP6 containing the full-length human LRP6 cDNA was kindly provided by Dr. Christof Niehrs (Deutsches Krebsforschungszentrum, Heidelberg, Germany). Plasmid pCS-VSVG-LRP6ΔC (hLRP6 mutant lacking cytoplasmic tail) was described

0014-5793/$32.00 
doi:10.1016/j.febslet.2006.09.011

before [22,24]. Human LRP6 mutant lacking the extracellular domain (LRP6ΔN) was constructed by PCR. This mutant is comprised human LRP6 signal peptide, 13 amino acid residues of the extracellular domain (four amino acid residues immediately after LRP6 signal peptide and nine amino acid residues immediately before the transmembrane domain), the transmembrane domain, and the entire cytoplasmic tail. The Myc epitope was inserted among 13 amino acid residues of LRP6 extracellular domain. The primers for PCR reaction are forward 5′-GATCGGATCCATGGGGGCCGTCCTGAGGAGCCTCCTGGCCTGCAGCTTCTGTGTGCTCCTGAGAGCGGCCCC TTTGTTGCTTGAACAAAAACTCATCTCAGAAGAGGATCTGGAAGAACCAGCACCACAGGCCAC-3′, and reverse 5′-GATCTCTAGATCAGGAGGAGTCTGTACAGGGAG-3′. The resulting PCR product was digested with *Bam*HI and *Xba*I, and ligated into pcDNA3 vector. The integrity of the construct was confirmed by sequence analysis. Mouse Mesd in TOPO pcDNA3.1/V5 His vector was obtained from Dr. Bernadette Holdener (State University of New York at Stony Brook, Stony Brook, NY).

### 2.2. Cell culture and transfection

HEK293 cells were cultured in Dulbecco's minimum essential medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and maintained at 37 °C in humidified air containing 5% $CO_2$. For transfection, cells were trypsinized and seeded into 6-well plates ($2.0 \times 10^5$ cells/well) without antibiotics. After 24 h, cells were transfected with various plasmids using FuGENE 6 (Roche) according to manufacturer's specifications. Cells were harvested for analysis 48 h after transfection.

### 2.3. Luciferase reporter assay

HEK293 cells were plated into 6-well plates. For each well, 0.1 μg of the TOP-FLASH luciferase construct (Upstate Biotechnology) was cotransfected with 0.1–1.6 μg of the full-length LRP6-expressing vector, LRP6 mutant expressing vector, Mesd expressing vector, or the empty pcDNA3 vector. A β-galactosidase-expressing vector (Promega, Madison, WI) was included as an internal control for transfection efficiency. After 48 h, cells were lysed, and luciferase activity was determines as described before [15].

### 2.4. Co-immunoprecipitation

Cells in 6-well plates were lysed with 0.5 ml of lysis buffer (phosphate-buffered saline containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride). Each aliquot of cell extracts (400 μg of protein) was brought to 0.5 ml with lysis buffer and mixed with 0.5 ml of phosphate-buffered saline containing 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 0.5% bovine serum albumin, and 1 mM phenylmethylsulfonyl fluoride (immunomixture), and then incubated with antibody for 2 h at 4 °C. Immunocomplexes were precipitated with protein A-agarose beads for 45 min, washed three times with immunomixture and two times with phosphate-buffered saline buffer, and boiled in SDS sample buffer containing β-mercaptoethanol. The supernatants were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting.

### 2.5. Western blotting

HEK293 cells transiently transfected with Myc-tagged LRP6, VSVG-tagged LRP6ΔC, or Flag-tagged Mesd were lysed with 0.5 ml of lysis buffer at 4 °C for 30 min. Equal quantities of protein were subjected to SDS–PAGE under non-reducing conditions. Following transfer to immobilon-P transfer membrane, successive incubations with anti-Myc antibody (Roche), anti-VSVG antibody (Roche), anti-Flag antibody (Sigma), or anti-actin antibody (Sigma), and horseradish peroxidase-conjugated secondary antibody were carried out for 60 min at room temperature. The immunoreactive proteins were then detected using the ECL system.

### 2.6. Cell surface ligand binding assay

Mouse recombinant Mesd protein was expressed and isolated as described previously [15]. Carrier-free $Na^{125}I$ was purchased from NEN Life Science Products. IODO-GEN was from Pierce. Mesd was iodinated by using the IODO-GEN method as described previously [15]. Cell surface $^{125}I$-Mesd binding assay was performed essentially as described [15].

## 3. Results

### 3.1. The full-length LRP6 is more efficient to induce Wnt signaling than its mutant lacking the extracellular domain

To fully understand the roles of LRP6 in Wnt signaling, we constructed a LRP6 mutant LRP6ΔN, which is comprised of LRP6 cytoplasmic tail, transmembrane domain, and only 13 amino residues of the extracellular domain (Fig. 1A). HEK293 cells in 6-well plates were transiently transfected with 0.2 μg of plasmid for the full-length LRP6 or LRP6ΔN, and Wnt signaling was analyzed by TOP-FLASH luciferase reporter assay. Interestingly, LRP6-transfected cells exhibited 12-fold greater TOP-FLASH luciferase activity than the control cells, whereas LRP6ΔN transfected cells did not display much change of the luciferase activity when compared to the LRP6 transfected cells (Fig. 1B). These results suggest that the full-length LRP6 is more efficient to induce Wnt signaling than the mutant lacking the extracellular domain. The strong LRP6-induced Wnt signaling is probably caused by the interaction between endogenous Wnt proteins and the receptor.

### 3.2. Dose-dependent increase in Wnt signaling induced by LRP6ΔN, but not the full-length LRP6

To further characterize the function of LRP6 and its mutant LRP6ΔN in Wnt signaling, we performed TOP-FLASH luciferase reporter assay after HEK293 cells were transiently trans-

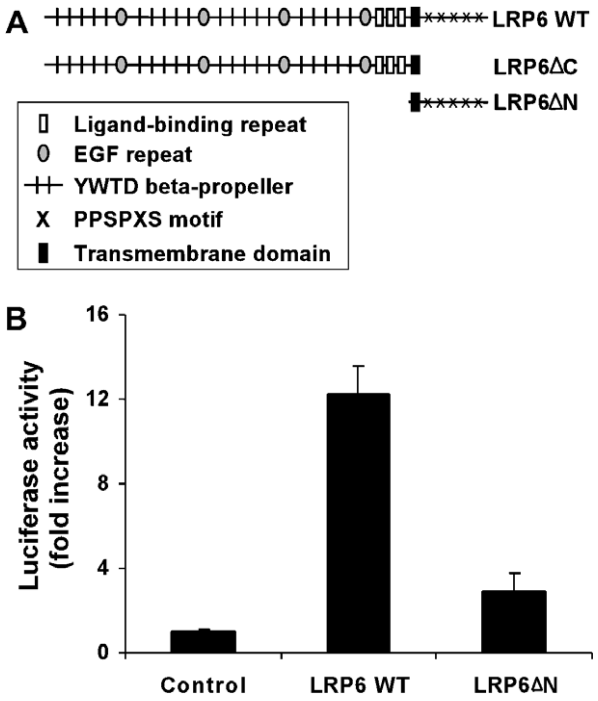


Fig. 1. The full-length LRP6 is more efficient to induce Wnt signaling than its mutant lacking the extracellular domain. (A) Schematic representation of the full-length LRP6 and its mutants. (B) Wnt-signaling induced by LRP6 and its mutant LRP6ΔN. HEK293 cells in 6-well plates were transfected with 0.2 μg of the full-length LRP6 expressing vector, LRP6ΔN expressing vector, or an empty vector, together with 0.1 μg of the TOP-FLASH luciferase construct and 0.1 μg of a β-galactosidase-expressing vector in each well. The luciferase activity was measured 48 hours after transfection. Values are the average of triple determinations with the S.D. indicated by error bars.

fected with different amounts of plasmid for LRP6 or LRP6ΔN. We found that Wnt signaling induced by LRP6ΔN was dose dependent, and that HEK293 cells transfected with 1.6 μg of LRP6ΔN plasmid exhibited a 23-fold greater activity than the control cells (Fig. 2). However, Wnt signaling induced by the full-length LRP6 did not display a dose dependent pattern. TOP-FLASH luciferase activity was slightly increased with the increased amounts of transfected LRP6 plasmid at first, but started to drop as the amounts of transfected LRP6 plasmid increased further. HEK293 cells transfected with 1.6 μg of LRP6 plasmid per well exhibited only a 10-fold greater activity than the control cells, which was significantly lower than the activity observed in HEK293 cells transfected with 0.4 μg of LRP6 plasmid per well (Fig. 2). These results suggest that a regulatory mechanism is in place to restrict the extent of LRP6-induced Wnt signaling, and that LRP6ΔN can escape this regulation.

### 3.3. Mesd promotes LRP6 expression on the cell surface and enhances Wnt signaling induced by LRP6

Mesd is a special chaperone for LRP5 and LRP6 [12–14]. As the Wnt signaling induced by LRP6 did not exhibit a dose dependent manner, we then studied the effect of Mesd expression on LRP6-induced Wnt signaling. As shown in Fig. 3A, with Mesd co-expression, Wnt signaling induced by LRP6 was gradually increased with the increasing amounts of LRP6 transfected into HEK293 cells. Consistent with previous studies [12–14], we found that the amounts of the LRP6 mature form were significantly increased in the presence of Mesd co-expression (Fig. 3B). It is generally believed that only cell surfaceLRP6 has function in inducing Wnt signaling [12–14,25]. In our previous study, we found that Mesd is a unique LRP6 ligand and binds to cell surface LRP6 with high affinity ($K_d \sim 3.3$ nM) [15]. To examine whether Mesd co-expression

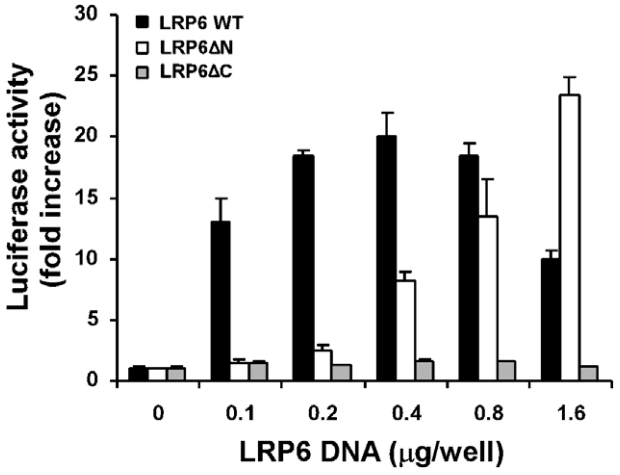


Fig. 2. Wnt signaling induced by overexpression of LRP6 mutant lacking the extracellular domain, but not of the full-length LRP6, exhibits a dose dependent manner. HEK293 cells in 6-well plates were transfected with 0.1–1.6 μg of the full-length LRP6 expressing vector, LRP6ΔN expressing vector, or LRP6ΔC expressing vector, together with 0.1 μg of the TOP-FLASH luciferase construct and 0.1 μg of a β-galactosidase-expressing vector in each well. The cells in each well were transfected with the same amounts of plasmid DNAs (1.8 μg/well) by balancing with the empty vector. The luciferase activity was measured 48 h after transfection. Values are the average of triple determinations with the S.D. indicated by error bars.

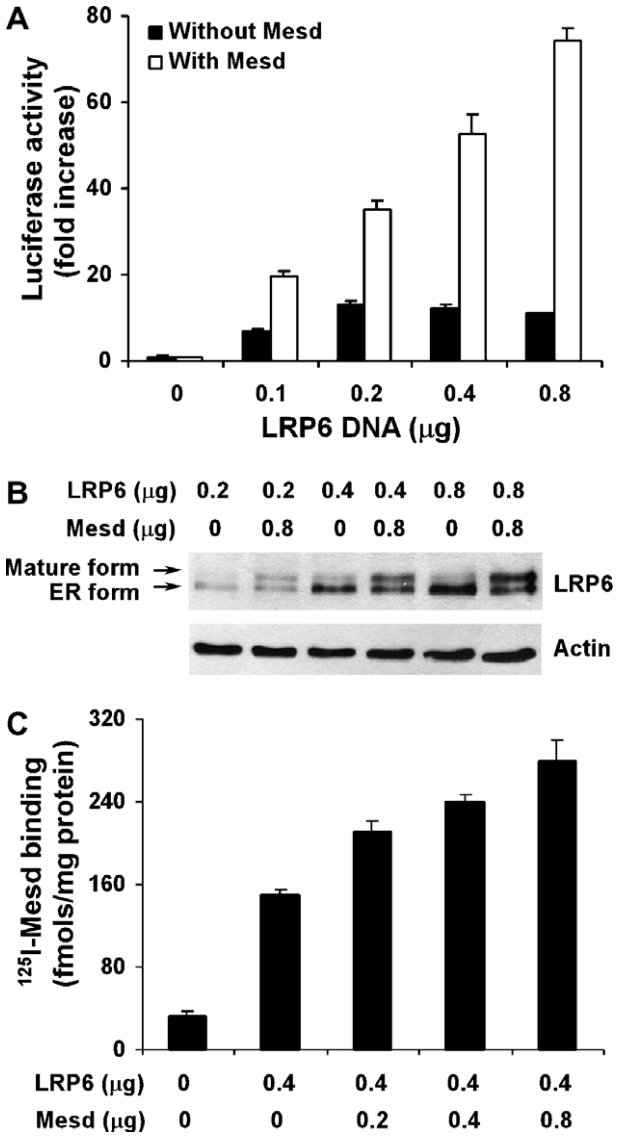


Fig. 3. Mesd promotes LRP6 expression on the cell surface and enhances Wnt signaling induced by LRP6. (A) Mesd enhances Wnt signaling induced by LRP6. HEK293 cells in 6-well plates were transiently transfected with LRP6 (0.1–0.8 μg/well), the TOP-FLASH luciferase construct (0.1 μg/well), and a β-galactosidase-expressing vector (0.1 μg/well), with the co-transfection of either empty pcDNA3 vector or pcDNA3-Mesd (0.8 μg/well). The cells in each well were transfected with the same amounts of plasmid DNAs (1.8 μg/well) by balancing with the empty vector. The luciferase activity was measured 48 h after transfection. Values are the average of triple determinations with the S.D. indicated by error bars. (B) Mesd promotes LRP6 maturation. HEK293 cells in 6-well plates were transiently transfected with LRP6 (Myc-tagged, 0.2–0.8 μg/well), with the co-transfection of either empty pcDNA3 vector, or pcDNA3-Mesd (0.8 μg/well). Forty-eight hours after transfection, the cells were lysed and the level of the full-length LRP6 was examined by Western blot analysis with anti-Myc antibody. The samples were also probed with an anti-actin antibody to verify equal loading. (C) Mesd promotes LRP6 expression on the cell surface. HEK293 cells in 6-well plates were co-transfected with LRP6 (0.4 μg/well) and Mesd (0.2–0.8 μg/well). The cells in each well were transfected with the same amounts of plasmid DNAs (1.2 μg/well) by balancing with the empty vector. Forty-eight hours after transfection, cells were incubated with $^{125}$I-Mesd (5 nM) at 4 °C for 3 h in the absence or the presence of 500 nM unlabeled Mesd. Cell surface bound $^{125}$I-Mesd was determined as described in Section 2. Values are the average of specific binding from triple determinations with the S.D. indicated by error bars.

promote LRP6 expression on the cell surface, we performed LRP6 ligand binding assay. We found that cell surface $^{125}$I-Mesd binding was gradually increased when LRP6 was co-transfected with increasing amounts of Mesd-expressing vector in HEK293 cells (Fig. 3C). Taken together, these results indicate that Mesd co-expression promotes LRP6 expression on the cell surface, which in turn enhances LRP6-induced Wnt signaling. We noted that Mesd co-expression induced a higher degree of the Wnt signaling increase (Fig. 3A) than the cell surface LRP6 expression change (Fig. 3B), suggesting that LRP6-induced Wnt signaling is amplified by other compounds of the Wnt signaling pathway.

### 3.4. Mesd can rescue Wnt signaling blocked by LRP6 mutant lacking the cytoplasmic tail

It has been reported that a LRP6 mutant lacking the intracellular domain is defective in Wnt signaling, and functions as a dominant-negative mutant [19–22]. Consistent with previous results, we found that LRP6ΔC, which encodes the entire extracellular domain and transmembrane domain, inhibited LRP6-induced Wnt signaling (Fig. 4A). In addition, we found that the level of the full-length LRP6 expression was gradually decreased with the increased amounts of LRP6ΔC plasmid co-transfected into HEK293 cells (Fig. 4B). We hypothesized that LRP6ΔC could impede LRP6 expression and function through sequestering LRP6 special chaperone Mesd. We then tested whether Mesd interacts with LRP6ΔC, and is required for LRP6ΔC folding and maturation. Co-immunoprecipitation results demonstrated that LRP6ΔC, like the full-length LRP6, interacts with Mesd (Fig. 5A). We also found that Mesd co-expression enhanced LRP6ΔC maturation (Fig. 5B). Furthermore, when HEK293 cells were co-transfected with Mesd, LRP6-induced Wnt signaling blocked by LRP6ΔC was partially rescued (Fig. 6A), and the expression of the full-length LRP6 mature form was partially restored too (Fig. 6B). Together, these data suggest that LRP6ΔC inhibits LRP6-mediated Wnt signaling by competing with LRP6 for binding to the folding chaperone Mesd.

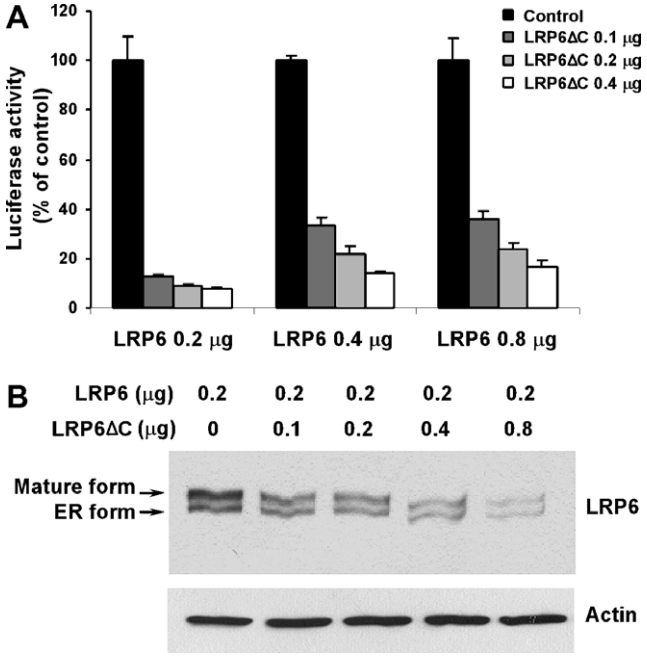


Fig. 4. LRP6 mutant lacking the intracellular domain blocks the full-length LRP6 expression and Wnt signaling. (A) LRP6ΔC blocks Wnt signaling induced by the full-length LRP6. HEK293 cells in 6-well plates were co-transfected with LRP6 (0.2–0.8 μg/well), LRP6ΔC (0.1–0.4 μg/well), the TOP-FLASH luciferase construct (0.1 μg/well), and a β-galactosidase-expressing vector (0.1 μg/well). The cells in each well were transfected with the same amounts of plasmid DNAs (1.4 μg/well) by balancing with the empty vector. The luciferase activity was measured 48 h after transfection. Values are the average of triple determinations with the S.D. indicated by error bars. (B) LRP6ΔC blocks the full-length LRP6 expression. HEK293 cells in 6-well plates were co-transfected with LRP6 (Myc-tagged, 0.2 μg/well), and LRP6ΔC (0.1–0.8 μg/well). The cells in each well were transfected with the same amounts of plasmid DNAs (1.0 μg/well) by balancing with the empty vector. 48 h after transfection, the cells were lysed, and the level of the full-length LRP6 was examined by Western blot analysis with anti-Myc antibody. The samples were also probed with an anti-actin antibody to verify equal loading.

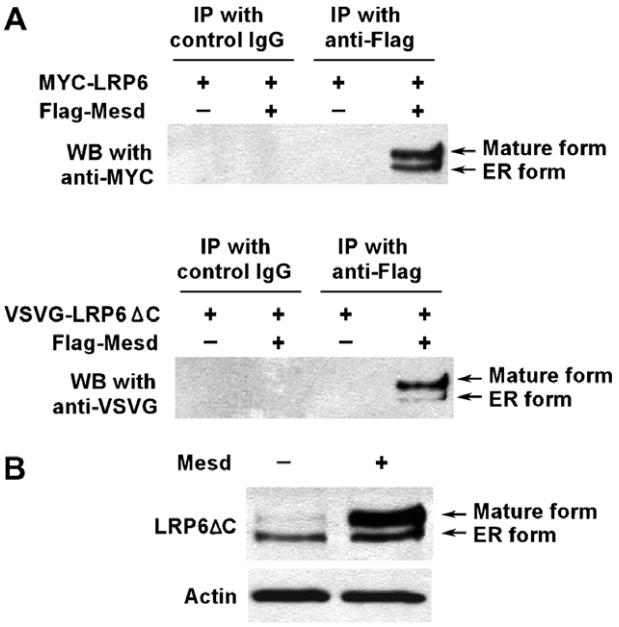


Fig. 5. Mesd interacts with LRP6ΔC and promotes LRP6ΔC maturation. (A) Mesd co-immunoprecipitates with LRP6ΔC. HEK293 cells in 6-well plates were transiently transfected with LRP6 (Myc-tagged, 1 μg/well) or LRP6ΔC (VSVG-tagged, 1 μg/well), with the co-transfection of either empty pcDNA3 vector, or pcDNA3-Mesd (Flag-tagged, 1 μg/well). Forty-eight hours after transfection, the cells were lysed and were immunoprecipitated with anti-Flag antibody or normal mouse IgG, and then probed with anti-Myc antibody to detect LRP6, or anti-VSVG antibody to detect LRP6ΔC. IP, immunoprecipitation; WB, Western blot. (B) Mesd promotes LRP6ΔC maturation. HEK293 cells in 6-well plates were transiently transfected with LRP6ΔC (VSVG-tagged, 0.6 μg/well), with the co-transfection of either empty pcDNA3 vector, or pcDNA3-Mesd (1.0 μg/well). Forty-eight hours after transfection, the cells were lysed and the LRP6ΔC expression was examined by Western blot analysis with anti-VSVG antibody. The samples were also probed with an anti-actin antibody to verify equal loading.

## 4. Discussion

The complex structures of the LDLR family members, including cysteine-rich repeats and β-propeller-like structures, determine that both general ER chaperones and those specialized for the LDLR family members are involved in promoting

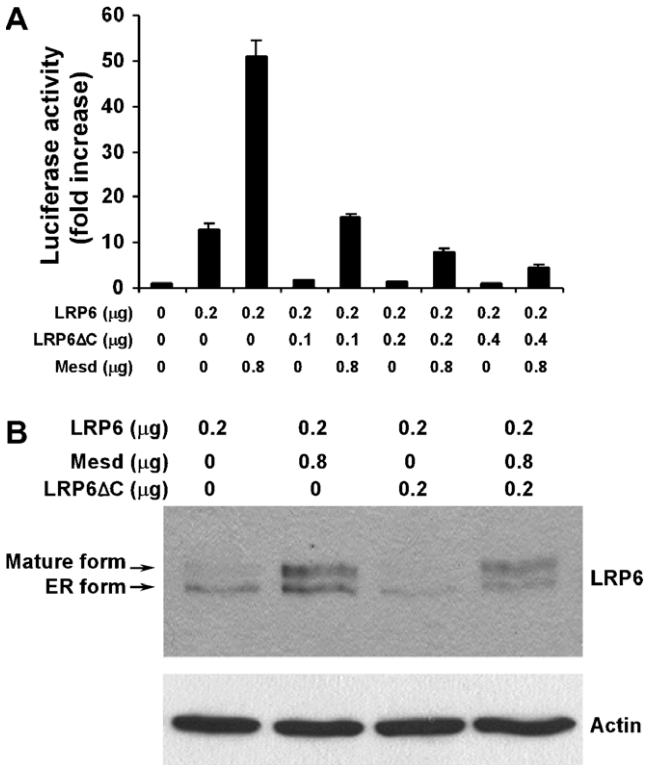


Fig. 6. Mesd can partially restore the full-length LRP6 expression and rescue Wnt signaling blocked by LRP6 mutant lacking the cytoplasmic tail. (A) Mesd rescues LRP6-induced Wnt signaling blocked by LRP6ΔC. HEK293 cells in 6-well plates were co-transfected with LRP6 (0.2 μg/well), LRP6ΔC (0.1–0.4 μg/well), Mesd (0.8 μg/well), the TOP-FLASH luciferase construct (0.1 μg/well), and a β-galactosidase-expressing vector (0.1 μg/well). The cells in each well were transfected with the same amounts of plasmid DNAs (1.6 μg/well) by balancing with the empty vector. The luciferase activity was measured 48 h after transfection. Values are the average of triple determinations with the S.D. indicated by error bars. (B) Mesd restores the full-length LRP6 expression. HEK293 cells in 6-well plates were co-transfected with LRP6 (Myc-tagged, 0.2 μg/well), LRP6ΔC (0.2 μg/well), and Mesd (0.8 μg/well). The cells in each well were transfected with the same amounts of plasmid DNAs (1.2 μg/well) by balancing with the empty vector. Forty-eight hours after transfection, the cells were lysed and the level of the full-length LRP6 was examined by Western blot analysis with anti-Myc antibody. The samples were also probed with an anti-actin antibody to verify equal loading.

their folding [11]. LRP5/6 has four β-propeller/EGF modules and three ligand binding repeats [4–6]. The formation of correct disulfide bonds during receptor folding presents a challenging task for ER chaperones. The function of Mesd (as well as RAP) during folding may be primarily to inhibit indiscriminate disulfide bond formation, in particular inter-molecularly between different receptor molecules during and after their translation [11]. Specifically, Mesd is required for the maturation of these β-propeller/EGF modules through the secretory pathway [12–14]. In the present study, we provided new evidence that Mesd is a crucial protein for Wnt signaling. We demonstrated that Mesd co-expression is required for LRP6 expression and function at the cell surface, and that LRP6 mutant lacking the cytoplasmic tail could impede LRP6 expression and function through sequestering Mesd protein.

RAP is another specialized molecular chaperone for the members of the LDLR family [11]. RAP binds with high affinity to most members of the LDLR family [11], but a lower affinity to LRP6 [15]. In the present study, we found that RAP co-expression had no significant effect on LRP6 folding and LRP6-induced Wnt signaling (data not shown), suggesting that RAP is not a critical specialized molecular chaperone for LRP6.

It is well established that RAP is a unique receptor antagonist for the LDLR family members. RAP can bind to cell surface receptors of the LDLR family, and is able to inhibit the binding of most currently known ligands of the LDLR family members [11]. Recently, we found that Mesd, like RAP, is capable of binding mature LRP6 at the cell surface and antagonizing ligand binding [15]. Depending on the nature of the LRP6 ligands, exogenously added recombinant Mesd protein can either upregulate or downregulate Wnt signaling. For example, Mesd can block the binding of Dickkopf 1 (Dkk1), a major secreted Wnt signaling antagonist [15]. In the present study, we provide new evidence that Mesd is a crucial protein for LRP6-mediated Wnt signaling at the cell surface. Thus, Mesd can modulate LRP6 activity by promoting its folding inside the cells and ligand binding capacity at the cell surface.

LRP6 constructs lacking their extracellular domain are able to activate the Wnt/β-catenin signaling in a Fz- and ligand-independent manner [9,19,23,24]. It was shown that a PPSPXS motif, which is reiterated five times in the LRP6 intracellular domain and is conserved between LRP5, LRP6, and their *Drosophila* homolog, Arrow, is necessary and sufficient to trigger Wnt/β-catenin signaling [24,26]. In the present study, we found that LRP6ΔN induced Wnt signaling in a dose-dependent manner (Fig. 2). LRP6ΔN lacks the complex structures such as cysteine-rich repeats and β-propeller-like structures, thus it is not surprising that Mesd co-expression is not required for LRP6ΔN signaling (data not shown). In the present study, we also found that the full-length LRP6 is more efficient to induce Wnt signaling than its mutant lacking the extracellular domain. Our data support the notion that both the extracellular domain and cytoplasmic tail of LRP6 are required for efficiently inducing Wnt signaling through LRP6, although the LRP5/6 mutants lacking the cytoplasmic tail exhibit inhibitory effects on the Wnt signaling [1].

The inhibitory activity of LRP5/6 mutant lacking the cytoplasmic tail is upstream in the Wnt/β-catenin pathway [19–22]. There might be several mechanisms by which this mutant serves as a dominant negative mutant for Wnt/β-catenin signaling. Firstly, the extracellular domain of LRP5/6 may work as an autoinhibitory domain [9,27]. It has been reported that the extracellular domain of the receptor might inhibit signaling by oligomerization [27]. Secondly, LRP5/6 mutant lacking the cytoplasmic tail could block Wnt signaling by sequestering LRP5/6 ligands Wnt proteins [22], as LRP5/6 mutants lacking the cytoplasmic tail are likely to retain the ability to bind LRP5/6 ligands. Finally, our present study indicates that sequestering special chaperone Mesd and impeding wild-type LRP6 expression could be the third and perhaps the most important mechanism by which the LRP5/6 mutant lacking the cytoplasmic tail to inhibit Wnt signaling in a dominant-negative fashion.

*Acknowledgements:* We are grateful to Drs. Bernadette C. Holdene and Christof Niehrs for providing the cDNAs of Mesd and LRP6. This work was supported in part by grant from the American Heart Association (0330118 N) to Y. L., and a grant from the National Institutes of Health (AG027924) to G.B.

## References


[1] He, X., Semenov, M., Tamai, K. and Zeng, X. (2004) LDL receptor-related proteins 5 and 6 in Wnt/β-catenin signaling: arrows point the way. Development 131, 1663–1677.

[2] May, P., Herz, J. and Bock, H.H. (2005) Molecular mechanisms of lipoprotein receptor signalling. Cell. Mol. Life Sci. 62, 2325–2338.

[3] Schneider, W.J. and Nimpf, J. (2003) LDL receptor relatives at the crossroad of endocytosis and signaling. Cell. Mol. Life Sci. 60, 892–903.

[4] Brown, S.D., Twells, R.C., Hey, P.J., Cox, R.D., Levy, E.R., Soderman, A.R., Metzker, M.L., Caskey, C.T., Todd, J.A. and Hess, J.F. (1998) Isolation and characterization of LRP6, a novel member of the low density lipoprotein receptor gene family. Biochem. Biophy. Res. Commun. 248, 879–888.

[5] Hey, P.J., Twells, R.C., Phillips, M.S., Nakagawa, Y., Brown, S.D., Kawaguchi, Y., Cox, R., Xie, G., Dugan, V., Hammond, H., Metzker, M.L., Todd, J.A. and Hess, J.F. (1998) Cloning of a novel member of the low-density lipoprotein receptor family. Gene 216, 103–111.

[6] Kim, D.H., Inagaki, Y., Suzuki, T., Ioka, R.X., Yoshioka, S.Z., Magoori, K., Kang, M.J., Cho, Y., Nakano, A.Z., Liu, Q., Fujino, T., Suzuki, H., Sasano, H. and Yamamoto, T.T. (1998) A new low density lipoprotein receptor related protein, LRP5, is expressed in hepatocytes and adrenal cortex, and recognizes apolipoprotein E. J. Biochem. 124, 1072–1076.

[7] Itasaki, N., Jones, C.M., Mercurio, S., Rowe, A., Domingos, P.M., Smith, J.C. and Krumlauf, R. (2003) Wise, a context-dependent activator and inhibitor of Wnt signalling. Development 130, 4295–4305.

[8] Li, X., Zhang, Y., Kang, H., Liu, W., Liu, P., Zhang, J., Harris, S.E. and Wu, D. (2005) Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. J. Biol. Chem. 280, 19883–19887.

[9] Mao, B., Wu, W., Li, Y., Hoppe, D., Stannek, P., Glinka, A. and Niehrs, C. (2001) LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. Nature 411, 321–325.

[10] Semenov, M., Tamai, K. and He, X. (2005) Sost is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. J. Biol. Chem. 280, 26770–26775.

[11] Bu, G. (2001) The roles of receptor-associated protein (RAP) as a molecular chaperone for members of the LDL receptor family. Int. Rev. Cytol. 209, 79–116.

[12] Culi, J. and Mann, R.S. (2003) Boca, an endoplasmic reticulum protein required for wingless signaling and trafficking of LDL receptor family members in Drosophila. Cell 112, 343–354.

[13] Hsieh, J.C., Lee, L., Zhang, L., Wefer, S., Brown, K., DeRossi, C., Wines, M.E., Rosenquist, T. and Holdener, B.C. (2003) Mesd encodes an LRP5/6 chaperone essential for specification of mouse embryonic polarity. Cell 112, 355–367.

[14] Culi, J., Springer, T.A. and Mann, R.S. (2004) Boca-dependent maturation of beta propeller/EGF modules in low-density lipoprotein receptor proteins. EMBO J. 23, 1372–1380.

[15] Li, Y., Chen, J., Lu, W., McCormick, L.M., Wang, J. and Bu, G. (2005) Mesd binds to mature LDL receptor-related protein-6 and antagonizes ligand binding. J. Cell Sci. 118, 5305–5314.

[16] Giles, R.H., van Es, J.H. and Clevers, H. (2003) Caught up in a Wnt storm: Wnt signaling in cancer. Biochim. Biophys. Acta 1653, 1–24.

[17] Moon, R.T., Kohn, A.D., De Ferrari, G.V. and Kaykas, A. (2004) WNT and beta-catenin signalling: diseases and therapies. Nat. Rev. Genet. 5, 691–701.

[18] Li, Y. and Bu, G. (2005) LRP5/6 in Wnt signaling and tumorigenesis. Future Oncol. 1, 673–681.

[19] Gong, Y., Slee, R.B., Fukai, N., Rawadi, G., Roman-Roman, S., Reginato, A.M., Wang, H., Cundy, T., Glorieux, F.H., Lev, D., Zacharin, M., Oexle, K., Marcelino, J., Suwairi, W., Heeger, S., Sabatakos, G., Apte, S., Adkins, W.N., Allgrove, J., Arslan-Kirchner, M., Batch, J.A., Beighton, P., Black, G.C., Boles, R.G., Boon, L.M., Borrone, C., Brunner, H.G., Carle, G.F., Dallapiccola, B., De Paepe, A., Floege, B., Halfhide, M.L., Hall, B., Hennekam, R.C., Hirose, T., Jans, A., Juppner, H., Kim, C.A., Keppler-Noreuil, K., Kohlschuetter, A., LaCombe, D., Lambert, M., Lemyre, E., Letteboer, T., Peltonen, L., Ramesar, R.S., Romanengo, M., Somer, H., Steichen-Gersdorf, E., Steinmann, B., Sullivan, B., Superti-Furga, A., Swoboda, W., van den Boogaard, M.J., Van Hul, W., Vikkula, M., Votruba, M., Zabel, B., Garcia, T., Baron, R., Olsen, B.R. and Warman, M.L.Osteoporosis-seudoglioma Syndrome Collaborative Group (2001) LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107, 513–523.

[20] Hoang, B.H., Kubo, T., Healey, J.H., Yang, R., Nathan, S.S., Kolb, E.A., Mazza, B., Meyers, P.A. and Gorlick, R. (2004) Dickkopf 3 inhibits invasion and motility of Saos-2 osteosarcoma cells by modulating the Wnt-beta-catenin pathway. Cancer Res. 64, 2734–2739.

[21] Mao, J., Wang, J., Liu, B., Pan, W., Farr 3rd, G.H., Flynn, C., Yuan, H., Takada, S., Kimelman, D., Li, L. and Wu, D. (2001) Low density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway. Mol. Cell 7, 801–809.

[22] Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J.P. and He, X. (2000) LDL receptor-related proteins in Wnt signal transduction. Nature 407, 530–535.

[23] Mi, K. and Johnson, G.V. (2005) Role of the intracellular domains of LRP5 and LRP6 in activating the Wnt canonical pathway. J. Cell. Biochem. 95, 328–338.

[24] Tamai, K., Zeng, X., Liu, C., Zhang, X., Harada, Y., Chang, Z. and He, X. (2004) A mechanism for Wnt coreceptor activation. Mol. Cell 13, 149–156.

[25] Cong, F., Schweizer, L. and Varmus, H. (2004) Wnt signals across the plasma membrane to activate the beta-catenin pathway by forming oligomers containing its receptors, Frizzled and LRP. Development 131, 5103–5115.

[26] Brennan, K., Gonzalez-Sancho, J.M., Castelo-Soccio, L.A., Howe, L.R. and Brown, A.M. (2004) Truncated mutants of the putative Wnt receptor LRP6/Arrow can stabilize beta-catenin independently of Frizzled proteins. Oncogene 23, 4873–4884.

[27] Liu, G., Bafico, A., Harris, V.K. and Aaronson, S.A. (2003) A novel mechanism for Wnt activation of canonical signaling through the LRP6 receptor. Mol. Cell. Biol. 23, 5825–5835.