Canadian Journal of Cardiology 35 (2019) 1567–1575

Review

Therapeutic Targeting of LRP6 in Cardiovascular Diseases: Challenging But Not Wnt-Possible!

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ABSTRACT

Coronary artery disease (CAD), often related to dyslipidemia, is a major cause of death worldwide, highlighting unmet therapeutic needs. Lipoprotein receptor-related protein 6 (LRP6) is a member of the low-density lipoprotein receptor (LDLR) family composed of structurally related cell surface receptors and acts, in consort with Frizzled receptors, as a coreceptor to mediate the Wnt/β-catenin signalling pathway. Impaired LRP6 signalling in humans has been associated with multiple cardiovascular risk factors such as elevated serum LDL, triglycerides, and glucose levels. Considerable efforts have been

RÉSUMÉ

La coronaropathie, souvent liée à la dyslipidémie, est une cause majeure de décès dans le monde qui témoigne de besoins thérapeutiques insatisfaits. La protéine 6 apparentée au récepteur des lipoprotéines de faible densité (LRP6) appartient à une famille de récepteurs de surface cellulaire structurellement apparentés appelés récepteurs des lipoprotéines de faible densité. La LRP6 intervient, de concert avec les récepteurs Frizzled, comme corécepteur dans la voie de signalisation Wnt/β-caténine. L'altération de la signalisation mettant en jeu la LRP6 chez l'être humain a été associée à de multiples

The family of low-density lipoprotein receptor (LDLR) is composed of cell surface proteins that are closely related structurally.[1] The first member of the family, the LDL receptor (LDLR), discovered in the early 1980s by Brown and Goldstein,[2] regulates cholesterol homeostasis by endocytosis of cholesterol-rich LDL particles. The second member, cloned in 1988, is the LDLR-related protein (LRP1), with close structural and biochemical similarities to the LDLR.[3] Several other LRPs were discovered thereafter (Fig. 1), including LRP6, cloned in 1998.[4,5] The LRPs share common structural elements[6]: (1) a ligand-binding domain composed of cysteine-rich repeats (between 2 and 11) that confers ligand specificity,[7] (2) epidermal growth factor (EGF) receptor-like domains, (3) YWTD (Tyr-Trp-Thr-Asp) β-propeller repeats that are involved in pH-dependent release of bound ligands after endocytosis and also may be involved in ligand binding,[8] and (4) a single-pass transmembrane domain (Fig. 1).

LRPs can be structurally subgrouped[9]: LDLR-related protein 1 (LRP1) and LRP2, also called glycoprotein 330 and megalin, are best known for their role as regulators of endocytosis and nervous system development[10]; LRP4 is crucial for the formation and stabilization of the neuromuscular junction[11]; and LRP8, also known as the apolipoprotein E receptor type 2 (ApoER2), is an important regulator of neuron migration during brain development.[12] LRP3, which plays a role in regulating osteogenic and adipogenic differentiation,[13] as well as LRP10 and LRP12, are structurally distant relatives and show atypical extracellular CUB domains (for Complement C1r/C1s, Uegf, BMP1).[6]

LRP6 was discovered to be part—together with LRP5 and the Frizzled receptors—of the 2 families of cell surface proteins involved in the mediation of Wnt/β-catenin signalling, acting as a coreceptor.[14] During the 1970s, genetic studies in *Drosophila melanogaster* identified a cluster of genes involved in a specific patterning phenotype during the development of the embryo. Among them were the "segment polarity genes," wingless, armadillo, and arrow, whose mutants showed specific patterning abnormalities.[15] Later, in the 1980s and 1990s, the vertebrate orthologues of the *Drosophila* genes—wingless, armadillo, and arrow—were identified, respectively, as int1/Wnt1, β-catenin, and LRP6, together with a significant number of other components of Wnt signalling from the cellular membrane to the nucleus (such as dishevelled, APC, GSK3, and Axin genes),[15] revealing Wnt as one of the most complex signalling pathways.

The protein encoded by the human LRP6 gene contains 1613 amino acids and is highly conserved among species.[16] It shares approximately 71% amino-acid identity with the protein encoded by LRP5 and is structurally similar: LRP5/6 show a unique organization among the LDLR family members, with 3

Received for publication June 7, 2019. Accepted June 28, 2019.

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See page 1573 for disclosure information.

https://doi.org/10.1016/j.cjca.2019.06.030

deployed to better understand the underlying mechanisms of LRP6-associated disorders, and the therapeutic targeting of LRP6 has been demonstrated to have positive effects in various animal models of cardiovascular disease. This review presents a synthetic summary highlighting the major roles of LRP6. LRP6 regulates a multitude of cellular mechanisms dependently or independently of the β-catenin pathway, as LRP6 activates gene transcription, regulates crucial cellular events such as cell cycle or protein synthesis, and even modulates gap junctional coupling in cardiomyocytes and LDLR recycling in hepatocytes. We discuss the potential contribution of LRP6 as a therapeutic target, as LRP6 inhibition limits myocardial fibrosis and promotes cardiac repair in myocardial infarction, limits neointimal formation in carotid injury models, decreases blood pressure in hypertensive animals, and reduces adipogenesis and lipogenesis to prevent hypercholesterolemia and atherosclerosis. These findings from past studies highlight LRP6 as a key player in the development of heart disease and a promising therapeutic target for cardiovascular disease in humans.

facteurs de risque cardiovasculaires, dont l'augmentation du taux de LDL sérique, du taux de triglycérides et de la glycémie. Des efforts considérables ont été déployés pour mieux comprendre les mécanismes sous-jacents aux troubles associés à la LRP6, et le ciblage thérapeutique de cette protéine a produit des effets positifs dans divers modèles animaux de maladies cardiovasculaires. Le présent article est un résumé synthétique soulignant les principaux rôles de la LRP6. La LRP6 régule une multitude de mécanismes cellulaires, indépendamment ou non de la voie de signalisation de la β-caténine. Ainsi, la LRP6 active la transcription génique, régule des événements cellulaires cruciaux tels que le cycle cellulaire ou la synthèse des protéines et elle module même le couplage au niveau des jonctions communicantes dans les cardiomyocytes et le recyclage du LDLR dans les hépatocytes. Nous abordons le rôle potentiel de la LRP6 en tant que cible thérapeutique, sachant que son inhibition limite la fibrose myocardique et favorise la réparation cardiaque en cas d'infarctus du myocarde, restreint la formation néointimale dans les modèles de lésions carotidiennes, produit un effet hypertensif chez l'animal et prévient l'hypercholestérolémie et l'athérosclérose en réduisant l'adipogenèse et la lipogenèse. Les résultats d'études antérieures que nous avons colligés mettent en lumière le rôle clé de la LRP6 dans l'étiologie cardiopathique de même que le caractère prometteur de cette protéine en tant que cible thérapeutique dans un contexte de maladie cardiovasculaire chez l'humain.

ligand-binding repeats and 4 EGF-type domains/YWTD repeats. Moreover, the cytoplasmic C-terminal domain of LRP5/6 includes 5 conserved PPPSP (Pro-Pro-Pro-Ser-Pro) motifs, contrary to the NPxY (Asn-Pro-any amino acid [x]-Tyr) motif(s) of other LDLR family members.[4] These PPPSP motifs have been demonstrated to be essential to trigger Wnt/beta-catenin signalling.[17]

Although initial studies mainly focused on the role of LRPs in the development of cancer, it has been gradually demonstrated that LRP6 plays also a major role in cardiovascular pathophysiology in humans; indeed, impaired LRP6 signalling has been associated with several risk factors for cardiovascular diseases including elevated serum LDL, triglyceride, and glucose levels.[18]

The contribution of other LRPs in cardiovascular pathophysiology is still not completely established. For example, LRP1 has been showed to both promote or protect against the development and progression of atherosclerosis.[19] Preclinical studies have identified LRP1 as a regulator of postinfarction remodelling.[20] Mutations in LRP2 were identified to cause embryonic heart development defects in mice.[21] LRP8 polymorphisms have been associated with a greater risk of premature atherosclerosis and acute myocardial infarction (MI) in humans,[22] but little is known about the underlying mechanisms. LRP5 also acts as a coreceptor to induce Wnt/β-catenin signalling and is (like LRP6) involved in cardiac disease; for example, LRP5 induces osteogenic differentiation in heart valve diseases in humans[23] and was recently demonstrated to play a protective role in the injured heart following MI in mice.[24]

However, even though LRP5 shares a similar structure and mediates Wnt signalling, previous studies showed that LRP5 and LRP6 have distinct roles owing to differences in tissue expression and affinity for specific Wnt ligands. LRP6 is much more efficient to induce dorsal axis duplication in *Xenopus* embryos[25] and mediates Wnt signalling transduction in response to different Wnt-Frizzled fusion proteins in 293T cells.[26] Thus, LRP6 qualifies as a major actor in cell signalling with specific properties and functions.

In this review, we chose to focus specifically on LRP6, its involvement in cardiovascular pathophysiology, and the promising results observed upon its therapeutic targeting in cardiovascular and metabolic diseases.

The Clinical Significance of LRP6 Malfunction as a Cardiovascular Risk Factor

In 2007, Mani et al.[27] reported a mutation on LRP6 (R611C) that causes precocious CAD associated with metabolic syndrome and low bone density in a family of Iranian ancestry. The same group[28] further showed that this variant both impairs LDL cholesterol clearance and the expression of the insulin receptor in peripheral tissues, an effect mediated by the Wnt canonical signalling pathway through TCF7L2, confirming the clinical significance of LRP6 as a regulator of metabolism and cardiovascular function. Interestingly, the mouse model $Lrp6R_{611C}$ displays a subtype of CAD that is not inflammatory, analogous to human forms of CAD that are not responsive to statins or anti-inflammatory therapies.[18] Since then, LRP6 genetic polymorphisms have been associated with many complex diseases, ranging from type 2 diabetes to neurodegenerative conditions.[29,30]

The multitude of cell mechanisms in which LRP6 is involved is illustrated in Figure 2. LRP6 can increase T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) transcriptional activity through the β-catenin complex to maintain cell proliferation,[31] control cell growth and division via Wnt/STOP signalling,[32] promote protein translation via Wnt/TOR signalling,[33] and interact with multiple G protein-coupled

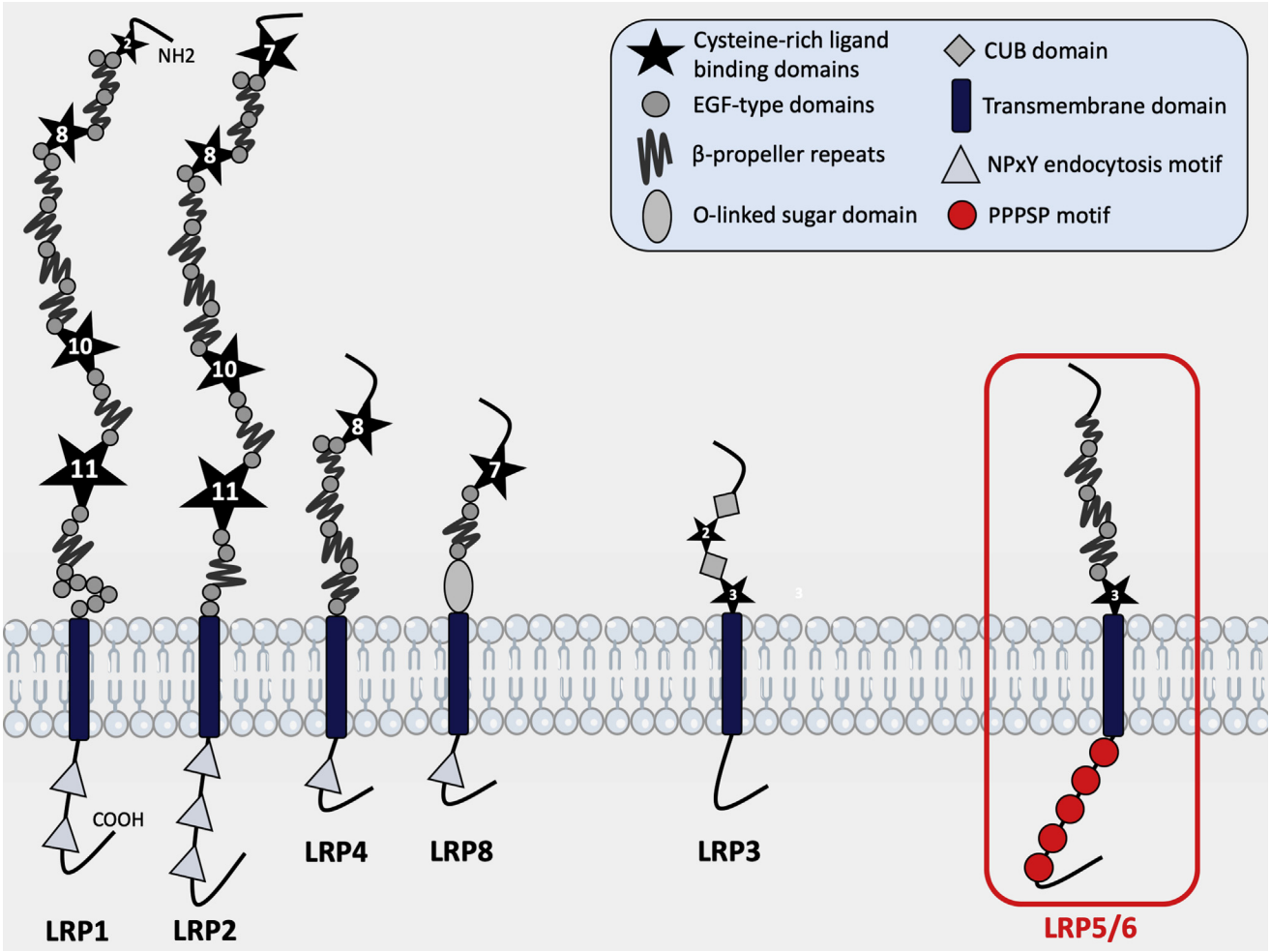

Figure 1. Schematic structure of the main LRP members. LRP5/6 show structural differences with the other members of the LRP family: a unique organization of 3 ligand-binding domains and 4 EGF-type domains and β-propeller repeats, as well as 5 PPPSP motifs. The number written in the **black stars** indicates the repetition of ligand-binding domains. CUB, Complement C1r/C1s, Uegf, BMP1; EGF, epidermal growth factor; NPxY, Asn-Pro-any amino acid (x)-Tyr; PPPSP, Pro-Pro-Pro-Ser-Pro. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

receptors (GPCRs).[34] LRP6 can also regulate noncanonical Wnt pathways, including the Wnt/PCP and Wnt/Ca^{2+} systems involved in the modulation of actin cytoskeleton and calcium signalling, respectively.[35,36] Notably, LRP6 can function independently of β-catenin: for example, by participating in the endocytosis of the LDLR/LDL-C complex in the liver or by regulating the gap junctional coupling of cardiomyocytes through control of connexin-43.[37,38] Because LRP6 governs many physiological phenotypes, it plays a crucial role in cardiac development, metabolism, and homeostasis and thus in the establishment and progression of heart disease.

Therapeutic Perspectives of LRP6-Targeting in Cardiovascular and Metabolic Diseases

Several approaches have been used to develop potentially useful LRP6 modulators and test them in animal models of cardiovascular and metabolic disease. A summary of these agents and their therapeutic perspectives for LRP6-related cardiovascular diseases is provided in Table 1.

Targeting LRP6 in animal models with biological and small-molecule inhibitors seems to be beneficial for the diseased myocardium and to offer interesting therapeutic potential. For example, GNF-6231, an inhibitor of porcupine (an endoplasmic reticulum protein required for Wnt palmitoylation), inhibits both canonical and noncanonical effects of Wnt ligands to limit the progression of myocardial fibrosis in a mouse model of myocardial infarction (MI).[39] The endogenous biological inhibitory secreted Frizzled related protein 5 (SFRP5) reduces canonical Wnt signalling to limit the development of myocardial inflammation in a mouse model of ischemia-reperfusion injury.[40] Another example of LRP6-targeting is the antagonism of tankyrase, a poly ADP-ribose polymerase that stimulates Axin degradation through the ubiquitin-proteasome system.[41] Because Axin is an endogenous inhibitor of the canonical Wnt pathway, therapeutic antagonists of tankyrase increase Axin levels; this has been shown to reduce formation of mechanical injury-induced neointima.[41] Dickkopf-related protein 1 and 2 (DKK1 and DKK2) are other therapeutic targets, as they both act as

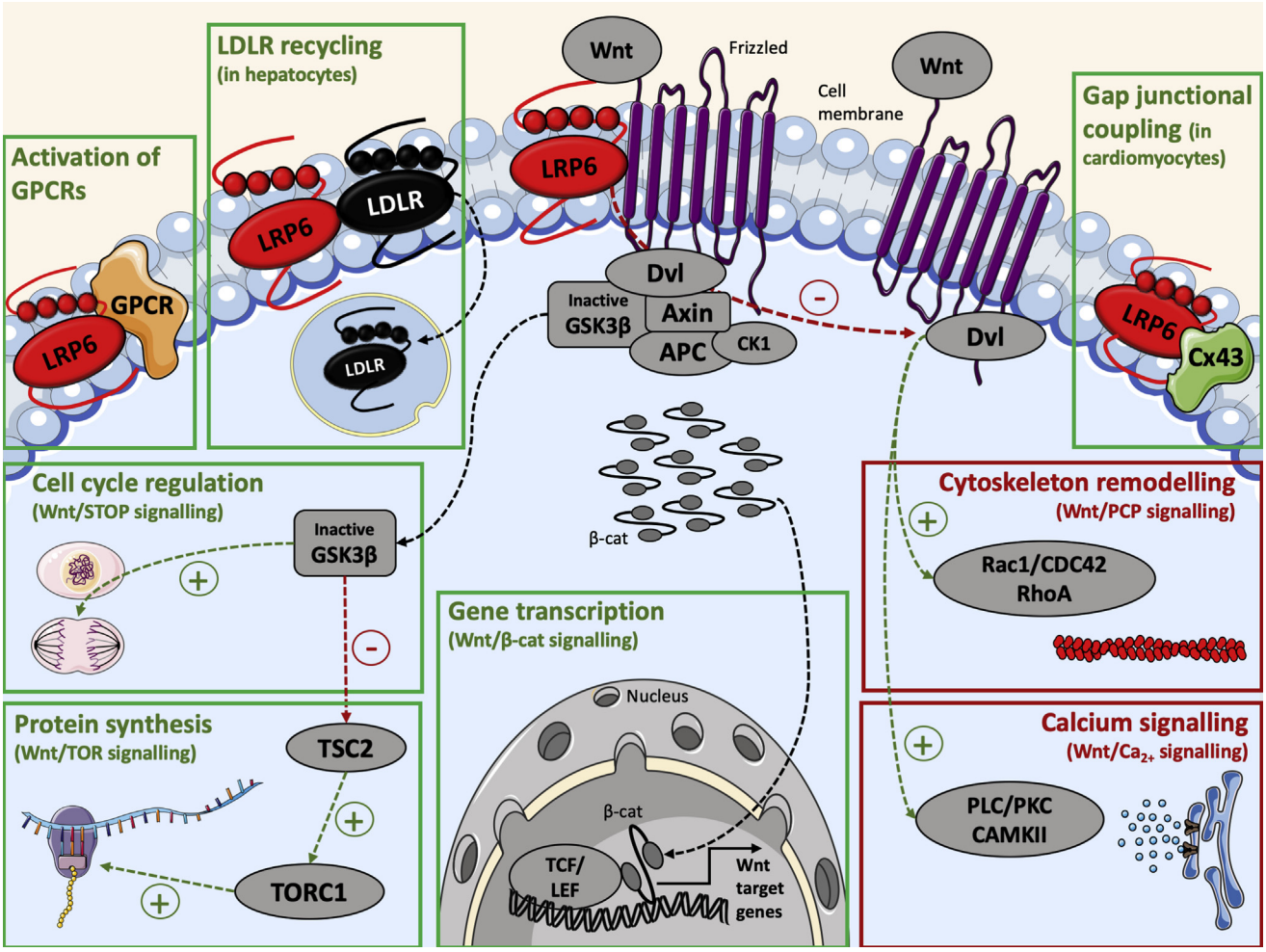

Figure 2. The multitude of LRP6 signalling pathways. LRP6 positively (in **green**) or negatively (in **red**) regulates a multitude of cellular mechanisms, dependently or independently, from the Wnt/β-catenin pathway. β-cat, β-catenin; APC, adenomatous polyposis coli; CAMKII, calcium/calmodulin-dependant protein kinase type II; CDC42, cell division control protein 42; CK1, casein kinase 1; Cx43, Connexin 43; DVL, dishevelled; GPCR, G protein-coupled receptor; GSK3β, glycogen synthase kinase 3 beta; LDLR, low-density lipoprotein receptor; LRP6, low-density lipoprotein receptor-related protein 6; PKC, protein kinase C; PLC, phospholipase C; RAC1, Rac family small GTPase 1; RhoA, ras homolog family member A; TCF/LEF, T-cell factor/lymphoid enhancer-binding factor; TORC1, rapamycin complex 1; TSC2, tuberous sclerosis complex 2. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

endogenous inhibitors of LRP6 activity: DKK1 regulates central blood pressure in hypertensive rats, and DKK2 improves angiogenesis and repair in rats with experimental MI.[42,43] On the other hand, modulation of JNK/LRP6 signalling by pharmacological inhibition of urotensin II receptors promoted cardiac progenitor cell proliferation and improved cardiac dysfunction in a mouse model of pressure overload, offering interesting perspectives for the treatment of heart failure.[44]

Interesting results have also been reported in studies targeting LRP6 in animal models of metabolic diseases. Notably, the micro-RNA miR-27a downregulates gene expression of LRP6 and LDLRAP1, decreasing the expression and the activity of the LDLR in the liver.[45] Coumestrol, a natural chemical present in plants, increases LRP6 protein expression and thus prevents adipogenesis by inhibiting adipocyte differentiation,[46] whereas the long noncoding RNA molecule LncRNA MEG3 competes with miR-21 to potentiate LRP6 expression, thus inhibiting hepatic lipogenesis.[47] The natural product toosendanin (TSN), which is the main active component of the fruit of *Melia toosendan* Sieb. et Zucc. (Meliaceae) used in traditional Chinese medicine, inhibits adipogenesis by activating Wnt/β-catenin signalling and increasing LRP6 mRNA levels.[48] The clinically relevant potential therapeutic effects of LRP6 modulation that have been observed in experimental models of cardiovascular and metabolic diseases are illustrated in Figure 3.

The Complexity of Targeting the Wnt Pathway for Therapeutic Purposes

Preclinical data clearly show that a range of molecules, including biologicals and small-molecule inhibitors, successfully target LRP6 itself or LRP6-dependant Wnt ligands to decrease blood pressure, limit myocardial inflammation and fibrosis, or ameliorate lipid metabolism (Table 1). However,

Table 1. Compounds targeting LRP6 pathways in preclinical development with potential clinical applications

	Compound	Animal or cellular models of disease	Target	Signalling pathway	Therapeutic effect	Reference
Cardiovascular diseases	GNF-6231	Myocardial infarction in C57BL/6 mice	Porcupine	Blocks secretion of Wnt ligands: reduction of extracellular matrix remodelling and myofibroblast proliferation	Limits fibrosis and reduces infarct size	[39]
	IGFBP-4	Myocardial infarction in C57BL/6 mice	LRP6 and Frizzled 8 (inhibits the binding of Wnt3a)	Prevents DNA damage: reduction of infarct size and hypertrophic genes levels	Limits fibrosis and reduces infarct size	[65]
	Dickkopf 2 (DKK2)	Myocardial infarction in Sprague-Dawley rats	LRP5/6	Induces angiogenesis; prevents myocardial cells death	Limits fibrosis and reduces infarct size	[43]
	Secreted Frizzled-related protein 5(SFRP5)	Ischemia/reperfusion injury in C57BL/6 mice	Wnt ligands; Frizzled	Reduces canonical Wnt signalling: reduction of inflammatory gene expression	Limits myocardial inflammation	[40]
	XAV939	Carotid artery ligation in C57BL/6 mice	Tankyrase	Reduces canonical Wnt signalling by increasing Axin levels: reduction of vascular smooth muscle cells (VSMCs) proliferation, migration and ROS release	Reduces intima formation	[66]
	miR-126-3p	Carotid artery injury in C57BL/6 mice; APOE-/- mice on high fat diet	LRP6	Reduces canonical Wnt signalling: reduction of VSMCs' proliferation and migration	Reduces intima formation	[67]
	circ_Lrp6	Carotid stenosis (collar placement) in $APOE^{-/-}$ mice	miR-145	circ_Lrp6 modulates miR-145 expression: viral delivery of circ_Lrp6 shRNA impairs VSMCs' proliferation and migration	Reduces intima formation	[68]
	Urantide	Transverse aorta constriction (TAC) in C57BL/6	Urotensin II receptor	Decreases JNK/ increases LRP6 signalling: increase of cardiac side population cells' (CSPCs') proliferation	Promotes cardiac repair and improves function	[44]
	Sclerostin (SOST)	Angiotensin II-infused $APOE^{-/-}$ mice	LRP5/6	Reduces canonical Wnt signalling: reduction of extracellular matrix remodelling	Prevents aortic aneurysm and atherosclerosis development	[69]
	Dickkopf 1 (DKK1)	Hypertensive rats (SHRs) and fructose-fed rats	LRP5/6	Increases GSK-3β activity; decreases IRS1 signalling; decreases NO levels	Modulates blood pressure	[42]
Metabolic diseases	miR-27a	HepG2 cells	LRP6 and LDLRAP1	Decreases LDLR levels; increases PCSK9 levels	Potential application for the treatment of hypercholesterolemia, atherosclerosis	[45]
	Coumestrol	Differentiated 3T3-L1 adipocytes	Estrogen receptor	Increases LRP6 and β-catenin expressions; decreases AKT and GSK3β activities	Inhibits adipogenesis	[46]
	Toosendanin (TSN)	Differentiated 3T3-L1 adipocytes; C57BL/6 mice on high fat diet	GSK3β and β-catenin	Enhances GSK3β phosphorylation; increases LRP6 mRNA levels; increases Wnt/β-catenin signalling by inhibiting β-catenin ubiquitination	Inhibits adipogenesis	[48]
	MEG3 (long noncoding RNA)	HepG2 cells	miR-21	Increases LRP6 expression; decreases AKT/mTOR signalling	Inhibits lipogenesis	[47]

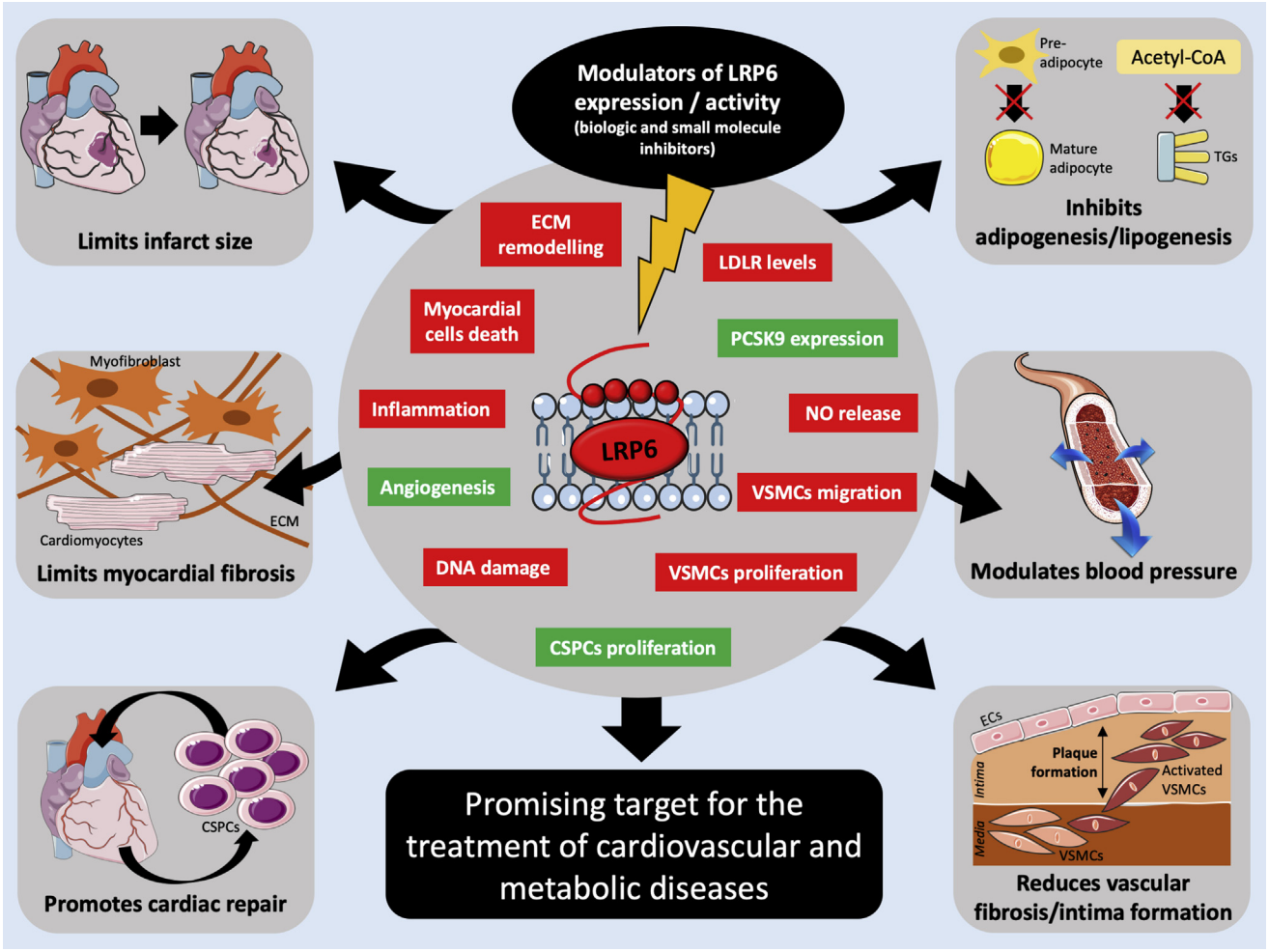

Figure 3. Therapeutic effects of LRP6 modulation in cardiovascular and metabolic diseases. Biologic or pharmacologic modulators of LRP6 expression and/or activity positively (in **green**) or negatively (in **red**) regulate cellular mechanisms to prevent the development of cardiovascular and metabolic diseases. Acetyl-CoA, acetyl coenzyme A; CSPCs, cardiac side population cells; ECM, extracellular matrix; ECs, endothelial cells; LDLR, low-density lipoprotein receptor; NO, nitric oxide; PCSK9, proprotein convertase subtilisin-kexin type 9; TGs, triglycerides; VSMCs, vascular smooth muscle cells. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

the question remains as to whether we can safely target LRP6-mediating Wnt signalling pathways in humans. Although extensive research in animal models has identified different potential pharmacological targets to treat aberrant Wnt signalling-related diseases, their validation in clinical trials is not so evident. In a review published in 2014,[49] Kahn underlined 3 factors making the clinical development of treatments specifically targeting the Wnt pathway very challenging: the multiple and subtle mechanisms of action of Wnt signalling (nuclear β-catenin levels, endogenous gene expression) that are mostly cell-type dependent; the multiple biological processes modulated, as nuclear β-catenin can bind to a wide range of transcription factors; and the temporality of the Wnt pathway, often finely regulated with an initial increase and a subsequent decrease in signalling (with negative feedback such as via Axin2, for example). Considering that LRP6 is involved in many cellular mechanisms, in multiple organs and cross-talk with other signalling pathways such as Notch, TGF-β/BMP, and free radical-generating pathways,[50] there are lots of hurdles in the development of drugs targeting the Wnt pathway without inducing adverse effects. For example, targeting LRP6 would not only result in the dysregulation of Wnt/β-catenin signals but also of GPCR signalling, which subserves extremely diverse functions. In addition, LRP6 can act independently of the β-catenin pathway.[51] Moreover, it has been demonstrated that proteolytic processing of LRP6 can drastically modulate its activity; for example, the intracellular C-terminal domains (membrane-anchored and cytosolic) of LRP6 are biologically active and increase TCF/LEF activation in the absence of Wnt3a.[52] On the other hand, LRP6 that lacks its extracellular domain can constitutively activate TCF/LEF and potentiate Wnt signalling.[53] Finally, Wnt ligands can also heterodimerize to create a unique biological effect.[54] Thus, developing a therapeutic biologically efficient molecule without toxic side effects is very difficult.

The complexity of therapeutically targeting the Wnt pathway is illustrated by past experience in cancer therapy. Depending on the type of cancer, Wnt signalling can either

promote or inhibit tumour initiation, and increased Wnt signalling can be both positively and negatively associated with patient outcomes.[55]

Current Status of Human Studies Targeting LRP6

Numerous modulators specifically targeting LRP6 have been tested *in vitro* with human preparations or cellular models of cancer.[55-57] For example, the loss of blood vessel epicardial substance (BVES)— a membrane protein involved in tight junctions regulation—in human-derived colonoids, increases LRP6 levels and activity and contributes to tumorigenesis, suggesting that upregulating BVES and thus decreasing LRP6 could prevent the development of colorectal cancer.[58] The treatment of human triple negative breast cancer cell lines with recombinant Mesd (a specific inhibitor of LRP6) decreases their migration and invasion.[59] Very recently, it has been demonstrated that incubation of Wnt-hypersensitive intestine mouse organoids with anti-LRP5/6 single-domain antibody fragment (VHHs) results in strong inhibition of Wnt pathway and thus the blocking of tumorigenic growth.[60] In addition, many other components tested *in vitro* also offer promising therapeutic perspectives for cancer therapy.[56] However, to the best of our knowledge, only a small number of therapeutic agents targeting various components of Wnt pathway—but not targeting specifically LRP6—have been successfully tested in phase 1 clinical trials with acceptable toxicity and are currently in phase 2 oncology trials.[56] Among them are PRI-724, developed by Prism Pharma and partnered with Eisai Pharmaceuticals, a compound that disrupts the interaction between β-catenin and the transcription coactivator CBP[61]; the Porcupine (an essential protein in Wnt secretion and activity) inhibitor LGK974/WNT974, developed by Novartis[62]; and the humanized anti-DKK1 antibody DKN-01 developed by Leap Therapeutics.[63] They have been used in patients with various malignancies such as melanoma and breast, colon, or liver cancer (https://clinicaltrials.gov). Other drugs in clinical evaluation have either displayed strong adverse effects such as fatigue, vomiting, abdominal pain, nausea, or bone fracture, or the results of these clinical studies have not yet been publicly disclosed.[49]

In the context of cardiovascular and metabolic diseases, despite the fact that most of the past studies have focused on the role of Wnt pathways in cancers,[55-57,64] there is a keen interest in identifying potentially useful modulators of Wnt signalling. Although promising therapeutic targets have been described in animal models, no therapeutic compound specifically targeting LRP6 has yet been developed. Considering the complexity of the Wnt pathways, the applicability of translating the targets identified in animal models into the clinical setting is far from being solved.

Conclusions

Wnt signalling plays a major role in the development and progression of heart disease by both altering metabolism (insulin sensitivity) and inducing structural changes (fibrosis, inflammation, hypertrophy).[54] Encouraging findings in recent years validate LRP6 as a promising therapeutic target for cardiovascular diseases, and efforts are being made to address how LRP6 could be safely, specifically, and effectively targeted in humans.

Funding Sources

Dr Thorin's laboratory is funded by the Canadian Institutes of Health Research (PJT-162446 and PJT-166110), The Natural Sciences and Engineering Research Council of Canada (Discovery funds, RGPIN-2017-04770), and the Foundation of the Montreal Heart Institute.

Disclosures

The authors have no conflicts of interest to disclose.

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