Non-canonical WNT signalling in cardiovascular disease: mechanisms and therapeutic implications

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Abstract | WNT signalling comprises a diverse spectrum of receptor-mediated pathways activated by a large family of WNT ligands and influencing fundamental biological processes. WNT signalling includes the β-catenin canonical pathway and the non-canonical pathways, namely the planar cell polarity and the calcium-dependent pathways. Advances over the past decade have linked non-canonical WNT signalling with key mechanisms of atherosclerosis, including oxidative stress, endothelial dysfunction, macrophage activation and vascular smooth muscle cell phenotype regulation. In addition, non-canonical WNT signalling is involved in crucial aspects of myocardial biology, from fibrosis to hypertrophy and oxidative stress. Importantly, non-canonical WNT signalling activation has complex effects in adipose tissue in the context of obesity, thereby potentially linking metabolic and vascular diseases. Tissue-specific targeting of non-canonical WNT signalling might be associated with substantial risks of off-target tumorigenesis, challenging its therapeutic potential. However, novel technologies, such as monoclonal antibodies, recombinant decoy receptors, tissue-specific gene silencing with small interfering RNAs and gene editing with CRISPR–Cas9, might enable more efficient therapeutic targeting of WNT signalling in the cardiovascular system. In this Review, we summarize the components of non-canonical WNT signalling, their links with the main mechanisms of atherosclerosis, heart failure and arrhythmias, and the rationale for targeting individual components of non-canonical WNT signalling for the treatment of cardiovascular disease.

Cardiovascular diseases, including atherosclerosis and myocardial disease, remain the leading cause of mortality worldwide[1]. Atherosclerosis is an inflammatory disease[2–4] influenced by genetic and demographic risk factors[5–7] and is associated with complex phenotypic changes in endothelial cells, vascular smooth muscle cells (VSMCs) and macrophages[8,9]. Myocardial disease is often associated with the presence of coronary atherosclerosis and involves processes such as myocardial oxidative stress[10,11], fibrosis and remodelling[12], which can lead to diseases such as heart failure and arrhythmias[11,13]. Despite substantial advances over the past decade, there is an unmet need to describe cardiovascular disease pathogenesis more accurately.

The WNT ligand family includes several secreted glycoproteins (19 in mammals) that activate an evolutionarily conserved spectrum of signalling pathways[14,15]. WNT signalling is implicated in embryogenesis and development via the regulation of cell motility and cell polarization. WNT signalling also regulates cell survival, growth and motility and has therefore been pathophysiologically linked with tumorigenesis[14,16] and cellular metabolism[17]. WNT signalling can be classified into two main modes of signalling: the canonical pathway, which was described first and involves a reduction in β-catenin degradation followed by the induction of the expression of β-catenin target genes, and the non-canonical pathway, which involves β-catenin-independent mechanisms such as Ca^{2+} signalling and activation of small GTPases[18]. WNT ligands have varying degrees of selectivity towards the two pathways and can activate either of the two pathways depending on spatiotemporal parameters and receptor availability[19]. Certain WNT ligands, such as WNT5A and WNT11, seem to predominantly activate non-canonical WNT signalling[15]. Non-canonical WNT ligands have been implicated in multiple biological processes such as inflammation, cell motility and metabolism[14]. WNT5A and WNT11 have even been proposed as markers of respiratory distress syndrome severity in patients with coronavirus disease 2019 (COVID-19)[20], which might be related to the capacity

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https://doi.org/10.1038/s41569-022-00718-5

Key points

- Cardiovascular disease is a major cause of morbidity and mortality worldwide, prompting the need for a better understanding of the underlying pathogenic mechanisms.
- Non-canonical WNT signalling involves an evolutionarily conserved and ubiquitous range of pathways affecting fundamental processes such as inflammation, metabolism, cell motility, oxidative stress and homeostasis.
- Non-canonical WNT signalling is a promising target in vascular disease, influencing vascular oxidative stress, endothelial dysfunction, inflammation, vascular smooth muscle cell phenotypes and cellular insulin resistance, all of which can affect atherosclerosis progression and plaque stability.
- Non-canonical WNT signalling has putative links to cardiac disease by influencing myocardial oxidative stress, inflammation, repair capacity, energetics and remodelling, including fibrotic or adipose infiltration of the myocardium, all of which can generate the substrate for contractile dysfunction and arrhythmogenic potential.
- Non-canonical WNT ligands are secreted by adipose tissue and are upregulated in obesity, acting as an endocrine and paracrine link between obesity and cardiovascular complications via adipose tissue–cardiovascular system crosstalk.
- Non-canonical WNT signalling can be therapeutically targeted at multiple levels and could involve several state-of-the-art technologies; however, more research in this area is required.

of these ligands to regulate cell migration, survival and apoptosis in lung injury. Additionally, non-canonical WNT signalling has emerged as a potential regulator of vascular disease pathogenesis via mechanistic links with inflammation, cell motility and oxidative stress[21–23]. Moreover, activation of non-canonical WNT signalling might be increased in obesity via the increased secretion of non-canonical WNT ligands, such as WNT5A, by adipose tissue[15].

In this Review, we explore the complex roles of non-canonical WNT signalling in cardiovascular disease. First, we provide an overview of WNT signalling, followed by a comprehensive description of the role of non-canonical WNT signalling in cardiovascular disease pathogenesis. Finally, we explore the clinical potential of targeting non-canonical WNT signalling to treat atherosclerosis.

WNT pathways and molecular targets

The prototype WNT signalling, currently referred to as canonical WNT signalling, was first described in *Drosophila*, where it was shown to be involved in wing formation by signalling mediated via the glycoprotein product of the *Wg* gene[24]. This gene was later found to be homologous to the mammalian gene *INT1*; therefore, the nomenclature for this family of *Wg*/*INT1* genes fused and became *WNT1*, and more WNT ligands have been described since[14,25]. In mammals, the WNT family of ligands consists of 19 glycoproteins (TABLE 1) that undergo a variety of post-translational modifications, including glycosylation and palmitoleic acid modifications[15,25]. As a result of these modifications, WNT ligands have moderate water solubility, which facilitates rapid protein–protein interactions and the propagation of paracrine signalling[25].

WNT ligands are ubiquitously secreted molecules without exclusive tissue sources. Pathophysiologically important sources include adipose tissue[15] and immune cells[23]. WNT ligands are also secreted by cardiovascular cells, including cardiomyocytes and the endothelium, although the baseline expression of WNT ligands in vascular cells is lower than in adipose tissue[15,26]. Nonetheless, the relative contribution of each tissue to the systemic WNT ligand pool has been poorly described. For example, WNT ligand release is a complex process involving post-translational modifications and vesicle secretion, and the upstream stimuli are unclear[27]. WNT ligands are often constitutively expressed and certain processes, such as inflammation and obesity, can upregulate their expression via mediators such as adiponectin[28].

WNT signalling is initiated after the binding of extracellular, secreted WNT ligands to various membrane receptors, mainly the Frizzled (FZD) family of G protein-coupled, seven-transmembrane receptors[14]. The tyrosine-protein kinase transmembrane receptors ROR1 and ROR2 and the tyrosine-protein kinase RYK have also been shown to interact with WNT ligands but these interactions are less well characterized than WNT–FZD interactions[29]. The WNT ligand–receptor interaction triggers an incompletely characterized chain of molecular events involving downstream protein interactions that lead to transcriptional regulation[14,25]. To date, two main pathways downstream of the WNT–receptor interaction have been described: the canonical WNT pathway and the non-canonical WNT pathway[29]. The canonical pathway prevents the degradation of β-catenin, thereby allowing β-catenin-dependent transcriptional regulation to occur, which affects cell proliferation and survival[30]. The non-canonical pathway involves β-catenin-independent downstream signalling and can be broadly divided into the planar cell polarity (PCP) pathway and the Ca^{2+}-dependent pathway[29]. WNT signalling is negatively regulated by the interaction of WNT ligands with secreted FZD-related proteins (SFRP1–SFRP5 in humans), which are structurally similar to FZD receptors and thus have affinity to WNT ligands and act as decoy receptors[29].

Of note, WNT ligands and receptors comprise an extremely complex canvas of interrelated pathways, and classification into canonical and non-canonical pathways and ligands is virtual and schematic. In reality, most WNT ligands might be able to activate both canonical or non-canonical WNT signalling, with the primary effects on one of the pathways depending on context, spatiotemporal parameters and receptor availability[19]. Moreover, a negative interaction between the canonical and non-canonical pathways has been described, suggesting continuous crosstalk between the various pathways and ligands[31,32].

Non-canonical WNT signalling

After the description of the canonical, β-catenin-dependent WNT signalling pathway, it became evident that certain WNT ligands exerted a wide range of biological effects in β-catenin-independent ways, namely the PCP and the Ca^{2+}-dependent pathway[33]. These non-canonical pathways are not fully characterized; however, certain relevant downstream mediators and phenotypic consequences have been identified[33] (FIG. 1).

The PCP pathway involves binding of a WNT ligand to FZD, ROR or RYK receptors[33]. This interaction leads to Dishevelled (DVL)-mediated GTP-dependent activation of small GTPases, such as RHOA and RAC1, although the exact intermediate events are not clear[29]. Activated RHOA and RAC1, in turn, stimulate the activation of JUN N-terminal kinase (JNK) via phosphorylation[34]. Given the involvement of RHOA and RAC1 in regulating cytoskeletal dynamics, changes in cell polarization and motility are the main phenotypes associated with activation of the PCP pathway[33,35]. In addition, WNT-mediated JNK activation has been linked with several other important pathophysiological processes such as inflammation and insulin resistance[34,36]. RAC1 can also influence oxidative stress via activation of NADPH oxidases[15], indicating that oxidative stress can be regulated by the PCP pathway.

WNT ligands can also trigger β-catenin-independent effects via regulation of intracellular Ca^{2+} concentration[33]. Indeed, WNT signalling acts in synergy with phospholipase C to increase intracellular Ca^{2+} levels[37], prompting activation of the Ca^{2+}-sensitive kinases protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase II (CaMKII)[33]. Ca^{2+}-mediated WNT signalling activates the Ca^{2+}-sensitive nuclear factor of activated T cells (NFAT) pathway[33], which is important in immune response regulation[38]. Ca^{2+}-dependent WNT signalling has mainly been explored in the context of embryonic development, and its clinical relevance in adults is less clear. However, considering the wide-ranging effects of its downstream targets PKC, CaMKII and NFAT, this mode of WNT signalling might contribute to several important biological processes[35].

Importantly, non-canonical WNT signalling is negatively regulated by canonical WNT signalling. The extracellular domain of LDL receptor-related protein 6 (LRP6), a membrane co-receptor of the canonical WNT signalling pathway, has been shown to interact physically with WNT5A, acting as a decoy receptor for non-canonical WNT signals[39]. In vivo, *Lrp6*$^{-/-}$ mice have congenital defects, which are rescued by *Wnt5a* deletion[39], suggesting that this LRP6-related phenotype is mediated by non-canonical WNT5A signalling. Similar defects were described in *Xenopus* embryos with knockdown of *lrp5* or *lrp6*, which were reversed by knockdown of the non-canonical WNT ligands *wnt5a* and *wnt11* (REF.[39]). WNT5A can activate or inhibit canonical WNT signalling in a spatiotemporal, tissue-specific manner[40], which could be a result of spatially different receptor and related regulatory co-receptor profiles, similar to LRP5 and LRP6. Non-canonical WNT effects could therefore occur indirectly via changes in canonical WNT dynamics.

Table 1 | **Overview of WNT ligands, receptors and associated pathways**

Protein	Involvement in signalling pathway	
	Canonical	Non-canonical
WNT ligands		
WNT1	++	+
WNT2	++	+
WNT2B	+	+
WNT3	++	+
WNT3A	++	+
WNT4	+	+
WNT5A	+	++
WNT5B	+	++
WNT6	+	+
WNT7A	+	+
WNT7B	+	+
WNT8A	++	+
WNT8B	+	+
WNT9A	+	+
WNT9B	+	+
WNT10A	++	+
WNT10B	++	+
WNT11	+	++
WNT16	+	+
Receptors		
FZD1	++	+
FZD2	+	++
FZD3	+	+
FZD4	+	+
FZD5	+	+
FZD6	+	++
FZD7	+	+
FZD8	+	+
FZD9	+	++
FZD10	+	++
LRP1, LRP5, LRP6	++	+
ROR1	+	++
ROR2	+	++
RYK	+	++

All ligands and WNT members can activate both pathways; assumed predominance of a given pathway is denoted as ++. FZD, Frizzled; LRP, LDL receptor-related protein; ROR, tyrosine-protein kinase transmembrane receptor ROR; RYK, tyrosine-protein kinase RYK.

Non-canonical WNT signalling in vascular diseases

Non-canonical WNT signalling has been implicated in key contributory factors to atherosclerosis (BOX 1), including oxidative stress, endothelial dysfunction, VSMC phenotypic switching and migration, and inflammation, as implied by observational association studies[29] and mechanistic evidence[15,23]. Of note, WNT5A is the best-studied non-canonical WNT ligand in cardiovascular disease to date, followed by other ligands such as WNT11 (REFS[23,29]). Therefore, most of the relevant studies used WNT5A as a representative ligand that primarily activates non-canonical WNT signalling in the

cardiovascular system. Nevertheless, the downstream pathways described in these studies could be activated by any WNT ligand in the appropriate circumstances, and WNT5A itself can also activate canonical WNT signalling in vascular cells[41,42].

Observational data

One of the first pieces of conclusive evidence for a causal link between WNT signalling and atherosclerosis came from a study published in 2007 on the genetic basis of the risk of early coronary artery disease (CAD) in a family with a high prevalence of CAD[43]. Genome-wide linkage analysis followed by direct DNA sequencing of the annotated genes and in vitro functional assessments demonstrated a link between the missense mutation R611C in *LRP6* and the early CAD phenotype[43], thereby causally linking WNT signalling with vascular disease. Further research has explored the mechanisms underlying this link, particularly regarding non-canonical WNT signalling.

Observational data indicate that high levels of circulating WNT5A are associated with the presence of atherosclerosis and related diseases such as obesity and diabetes mellitus[15,23,44]. Work from our group has demonstrated that, in humans, CAD is associated with elevated WNT5A bioavailability in the plasma independently of demographic risk factors[15]. In addition, in patients who underwent two CT scans for coronary artery calcium score quantification, plasma WNT5A levels were positively associated with calcified plaque burden and new-onset calcification, independent of traditional risk factors[15]. Furthermore, WNT5A is locally expressed in mouse and human atherosclerotic plaques[21]. Levels of the non-canonical ligands WNT5A, WNT5B and WNT11 are also upregulated in human aortic calcified valves compared with non-calcified valves[45]. Given the link between these ligands and non-canonical activation of osteogenesis-related pathways in a variety of in vitro models[46], non-canonical WNT might be hypothesized to contribute to valve or vascular wall calcification.

The aforementioned observational findings provide strong proof of concept for a link between non-canonical WNT signalling (mainly WNT5A) and atherosclerosis[47] but observational results do not confirm causality. However, consistent with the observational findings, several experimental studies have revealed underlying cellular hubs through which non-canonical WNT signalling causally interacts with key mechanisms of atherosclerosis (FIG. 2).

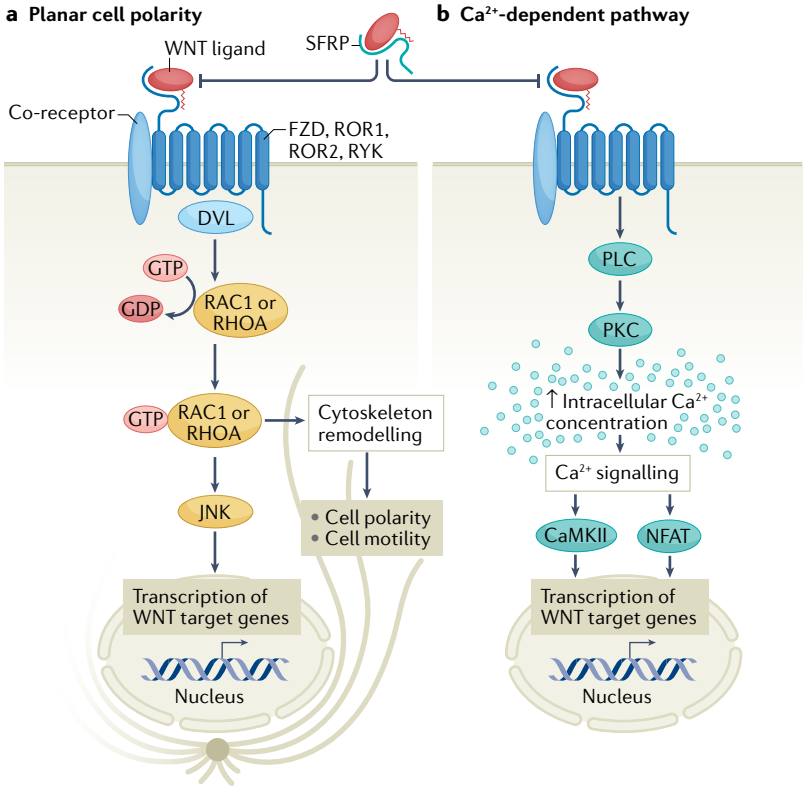

Fig. 1 | **Overview of non-canonical WNT signalling pathways.** Non-canonical WNT signalling pathways involve the planar cell polarity pathway (part **a**) and the Ca^{2+}-dependent pathway (part **b**). Both pathways are initiated by binding of a WNT ligand to WNT receptors, such as Frizzled (FZD) receptors, the tyrosine-protein kinase transmembrane receptors ROR1 and ROR2, and tyrosine-protein kinase RYK, which belong to the family of G protein-coupled receptors (also known as seven-transmembrane receptors), with the potential contribution of various co-receptors. Binding of WNT ligands to secreted FZD-related proteins (SFRPs) blocks the WNT–receptor interaction. The downstream events involved in the planar cell polarity pathway are not well defined, but include Dishevelled (DVL) and lead to GTP-dependent activation of small GTPases, such as RAC1 and RHOA, which in turn activate JUN N-terminal kinase (JNK), and ultimately regulate cell polarity and motility and gene transcription. The Ca^{2+}-dependent pathway involves activation of PLC and protein kinase C (PKC), which leads to increased intracellular Ca^{2+} concentration, triggering the activation of calcium/calmodulin-dependent protein kinase II (CaMKII) and the nuclear factor of activated T cells (NFAT) pathway, leading to transcriptional regulation.

Oxidative stress

WNT5A signalling increases the production of reactive oxygen species (ROS) in the human granulosa-like tumour cell line KGN, which was speculated to be related to the induction of inflammation considering that lipopolysaccharide stimulation had the same effect on ROS production in these cells[36]. Despite the incomplete mechanistic data, this observation supports the notion that WNT5A might directly increase ROS production. By contrast, WNT5A has been shown to protect against oxidative stress-induced VSMC apoptosis, although this effect was achieved with a supraphysiological concentration of WNT5A that cross-activated the canonical pathway[42]. Therefore, this finding should be regarded as being mediated by canonical WNT signalling rather than a specific WNT5A-mediated effect.

Work from our group was the first to directly explore the role of WNT5A in the regulation of vascular oxidative stress in the context of atherosclerosis[15]. After demonstrating the specificity of physiological WNT5A concentrations towards non-canonical signalling, we showed that WNT5A directly increased NADPH oxidase activity in vitro in arteries from patients with CAD[15]. This effect was mediated via increased GTP-dependent activation and membrane translocation of RAC1 (which is both a downstream target of the PCP pathway and a key subunit of the NADPH oxidase isoforms NOX1 and NOX2)[15]. This finding was further validated in vitro in WNT5A-treated primary VSMCs from patients with CAD as well as in aortic segments

Box 1 | **Fundamental mechanisms of atherosclerosis**

Atherosclerosis is a complex disease in terms of its underlying mechanisms. Disease initiation has long been thought to occur at endothelial sites that are subjected to dysregulated shear stress such as the coronary arteries and other sites prone to developing turbulent flow (such as vascular bifurcations)[8]. Pulsatile, turbulent shear stress results in endothelial dysfunction and disruption of endothelial barrier integrity[177], resulting in the deposition of lipids, such LDL, in the subendothelial space[8,178]. LDL can then be oxidized as a result of various pro-oxidant stimuli such as local inflammation (secondary to local tissue damage)[178]. The oxidized LDL is internalized by macrophages, leading to macrophage activation and transformation into foam cells and further promoting inflammation and oxidative stress[178]. This vicious cycle establishes and promotes the formation and progression of atherosclerotic plaques.

Several pathogenic mechanisms are involved in promoting the atherogenic cycle, inducing further LDL oxidation, endothelial cell activation, macrophage recruitment and activation, and vascular smooth muscle cell phenotypic switch[178,179], ultimately influencing atherosclerotic plaque characteristics and overall plaque burden[180]. Oxidative stress is one such mechanism, characterized by the overproduction of reactive oxygen species by enzymes such as NADPH oxidases and uncoupled endothelial nitric oxide synthase[6]. The overproduction of reactive oxygen species in turn leads to reduced nitric oxide bioavailability and endothelial dysfunction[6]. Local inflammation and cellular insulin resistance at the level of the vascular wall are additional mechanisms contributing to atherosclerotic plaque burden via modulation of vascular smooth muscle cell phenotype and promotion of lipid oxidation and plaque necrotic core remodelling[5,159,180,181].

from mice overexpressing WNT5A[15]. Importantly, transcriptome analysis of WNT5A-treated human primary VSMCs revealed that WNT5A induces the differential expression of a large number of genes, and the expression profile of several of these genes was restored by administration of pegylated superoxide dismutase, suggesting that WNT5A is involved in redox-dependent transcriptional regulation in human vascular tissue[15]. Finally, we identified the ubiquitinating enzyme USP17 as a redox-sensitive downstream target of WNT5A that increases the GTP-dependent activation of RAC1 (REF.[15]). This study was the first to establish a causal role of WNT5A in increasing oxidative stress in human atherosclerosis and identifies USP17 and RAC1 as the key mediators of WNT5A-induced oxidative stress.

In contrast to our findings, other work suggests that WNT5A inhibits hydrogen peroxide (H_2O_2)-induced apoptosis in VSMCs via CCN family member 4 (also known as WISP1), an effect mediated through canonical WNT signalling[42]. This finding could suggest that WNT5A has an antioxidant function; however, WNT5A was used at a supraphysiological concentration in this study, which might mean that this canonical WNT signalling effect on H_2O_2-induced apoptosis might not be physiological. Conversely, H_2O_2 is a more stable type of ROS than superoxide and has subtler signalling roles[42]. As such, WNT5A-mediated signalling might theoretically stimulate the production of both superoxide (which could be detrimental when in excess) and H_2O_2 (which could provide a more prolonged signalling effect), hinting towards a delicate WNT5A-regulated redox balance, which warrants further investigation.

Given that the activation of endothelial nitric oxide synthase (eNOS) is partly mediated by CaMKII[48] and that both PKC and CaMKII interact with NADPH oxidases[49–51], it is highly plausible that non-canonical WNT Ca^{2+} signalling also regulates vascular oxidative stress and thereby contributes to atherosclerosis. However, this hypothesis remains to be studied.

Endothelial dysfunction

Although canonical WNT signalling has been linked to endothelial cell survival and angiogenesis[52], the role of non-canonical WNT signalling in endothelial cells has been explored only during the past decade. A study in primary endothelial cells from patients with diabetes revealed that WNT5A signalling impairs AKT phosphorylation, eNOS activity, nitric oxide (NO) bioavailability and, ultimately, endothelial function in vitro, mediated by non-canonical JNK-mediated signalling[53]. Consistent with this finding, our group demonstrated that WNT5A-mediated signalling directly impairs NO bioavailability and endothelial function, evidenced by ex vivo endothelium-dependent vasorelaxation of WNT5A-incubated artery samples from patients with CAD[15]. This reduced NO bioavailability was secondary to NADPH oxidase activation by WNT5A, as explained in previous sections, which led to oxidative depletion of tetrahydrobiopterin[15]. The absence of tetrahydrobiopterin resulted in eNOS uncoupling and production of superoxide instead of NO[15].

These findings provide proof of the causal role of WNT5A in propagating a dysfunctional endothelial phenotype in humans. However, the underlying mechanisms for this role have not been fully explored. In addition, as mentioned in the previous section, Ca^{2+}-dependent WNT signalling potentially affects both eNOS coupling and activity via CaMKII and PKC[48,54–56] and could, therefore, be hypothesized to contribute to endothelial dysfunction, which warrants further investigation.

VSMC function and phenotype

Cell motility, migration and polarity are the best-studied phenotypes regulated by non-canonical WNT signalling, which has been demonstrated in a variety of embryonic development models and studies of tumours or cell types such as erythrocytes[23,57–59]. Canonical WNT signalling has been linked to VSMC biology, particularly cell migration[42,60,61]. However, evidence also suggests roles for non-canonical signalling in VSMCs.

After the demonstration of a link between the *LPR6* R611C variant and CAD, further research has shed light on the underlying mechanisms with the use of VSMC in vitro assays and transgenic mouse models[62]. Indeed, loss of normal LRP6 activity as a result of the R611C variant is associated with increased non-canonical WNT signalling and is evidenced by increased RHOA and JNK activity[62]. The activation of non-canonical WNT signalling resulted in the activation of serine/threonine-protein kinase NLK followed by phosphorylation and subsequent ubiquitylation and degradation of transcription factor 7-like 2, a transcription factor that is associated with canonical WNT signalling[62]. These signalling events were associated with a phenotypic switch in VSMCs from a contractile to a synthetic phenotype, arterial media thickening and CAD promotion in mice[62]. Exogenous treatment with the canonical ligand WNT3A reversed the effects of the LRP6-R611C variant[62]. This finding is an elegant example of the crosstalk between the canonical and non-canonical pathways, with reciprocal effects on VSMC phenotypes.

Our group has shown that WNT5A induces redox-sensitive migration of human primary VSMCs without affecting their proliferation[15]. This effect could be partly mediated by RAC1 activation, but transcriptome analysis showed that WNT5A-mediated signalling also affected the expression of multiple genes related to migration in primary human VSMCs[15]. In addition, WNT5A can inhibit oxidative stress-induced apoptosis of VSMCs via the induction of WISP1 (REF.[42]). However, this effect was shown to be mediated by β-catenin signalling[42] and might not be relevant in vivo given that WNT5A, at physiological concentrations, is a selective ligand for non-canonical WNT signalling. Another non-canonical WNT ligand, WNT4, has been shown to stimulate VSMC proliferation, whereas its downregulation attenuates intima–media thickening in mice[63].

Beyond its effects on VSMC migration, non-canonical WNT signalling has been shown to induce a phenotypic switch in human primary VSMCs characterized by the loss of expression of the contractile markers aortic smooth muscle actin (also known as α2-SMA) and transgrelin, and an increased expression of matrix metalloproteinase 9 (MMP9)[15]. MMP9 has been shown to induce destabilization of atherosclerotic plaques in mouse models of atherosclerosis and in human genetics studies[64–67]. Taken together, these findings suggest that non-canonical WNT signalling might promote an 'aggressive' phenotype in VSMCs, characterized by an increased propensity for migration, the switch to a less differentiated phenotype and the upregulation of potentially plaque-destabilizing MMPs. WNT5A has been reported to be among the WNT ligands expressed by VSMCs isolated from artery samples from patients undergoing coronary artery bypass graft surgery, which might be related to neointima formation[68].

Non-canonical WNT signalling can also influence atherosclerotic plaque calcification by inducing changes in VSMC biology. WNT5A was found to stimulate the expression of genes related to chondrogenesis in both mouse and human VSMCs[69]. WNT5A-mediated gene expression was inhibited by peroxisome proliferator-activated receptor-γ (PPARγ) signalling via SFRP2, suggesting that WNT5A promotes vascular calcification[69]. In addition, a correlation has been observed between the WNT5A–ROR2 pathway and the degree of VSMC calcification in vitro[70]. Canonical WNT signalling has also been shown to regulate vascular calcification in mouse aorta via bone morphogenetic protein 2 signalling[46]. Interestingly, co-expression of the non-canonical WNT receptor FZD10 and the canonical co-receptors LRP5 or LRP6 in VSMCs leads to cross-inhibitory signals between the two pathways[71]. By contrast, LPR5 or LRP6 loss of function increased non-canonical WNT signalling and inhibited canonical β-catenin signalling, which was associated with a shift towards osteochondrogenic programming and calcification in VSMCs[71]. These findings suggest a competition between the canonical and non-canonical pathways with regard to VSMC calcification, with LRP5 and LRP6 at the core.

Non-canonical WNT signalling can affect intracellular cholesterol accumulation. In an *Apoe*$^{-/-}$ mouse model of atherosclerosis, in vivo adenovirus-mediated delivery to aortic tissues of small interfering RNA targeting *Wnt5a* reduced atherosclerotic plaque lipid content without affecting blood lipid levels[36]. WNT5A has also been suggested to facilitate foam cell formation[23]. By contrast, another in vitro study indicated that WNT5A reduces intracellular cholesterol in VSMCs treated with oxidized LDL mediated by stimulating the expression of

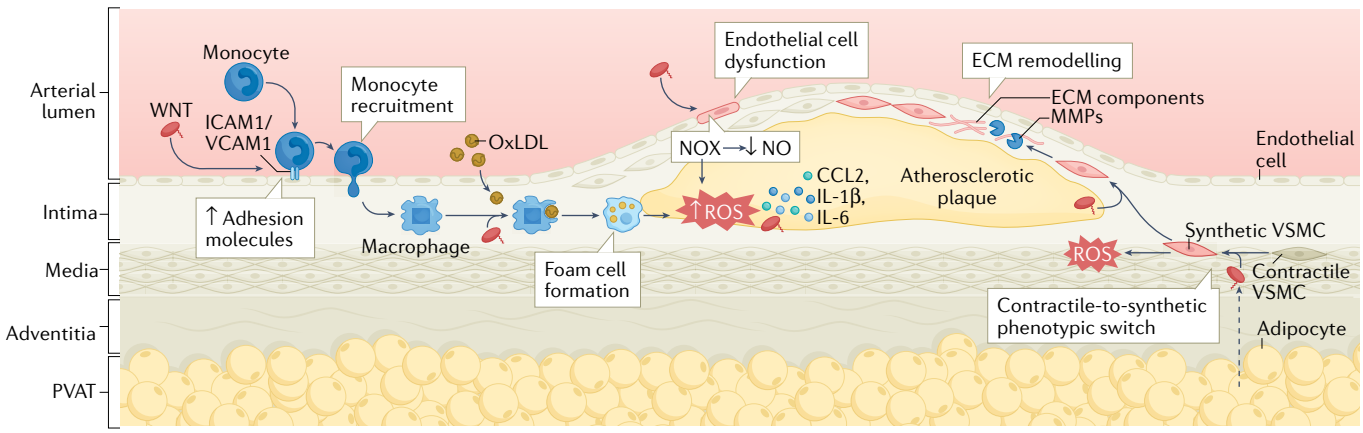

Fig. 2 | **Non-canonical WNT signalling in atherosclerosis.** Non-canonical WNT ligands, such as WNT5A, can enter the vascular wall from the circulation and are also released by vascular macrophages and the perivascular adipose tissue (PVAT). In endothelial cells, binding of non-canonical WNT ligands to their receptors leads to the upregulation of the expression of intracellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), which promotes monocyte recruitment to the arterial intima. Non-canonical WNT signalling in endothelial cells also induces the activation of NADPH oxidases (NOX), which leads to increased production of reactive oxygen species (ROS), activation of pro-inflammatory redox signalling, reduced nitric oxide (NO) bioavailability and endothelial dysfunction. In monocytes and macrophages, non-canonical WNT signalling promotes pro-inflammatory activation, uptake of oxidized LDL (oxLDL) and foam cell formation, and production of pro-inflammatory cytokines such as C-C motif ligand 2 (CCL2), IL-1β and IL-6. In vascular smooth muscle cells (VSMCs), non-canonical WNT signalling induces a switch from a contractile to a synthetic phenotype and increases cell migration via ROS signalling, thereby promoting the migration of synthetic VSMCs into the atherosclerotic plaque, where they produce extracellular matrix (ECM) components and matrix metalloproteinases (MMPs). These mechanisms interact in complex ways to establish a vicious cycle, directly promoting atherogenesis and plaque instability.

ATP-binding cassette transporter 1 (ABCA1)[72], which is involved in reverse cholesterol transport. Overall, non-canonical WNT signalling seems to be involved in lipid handling in atherosclerotic plaque cells, but the exact effects and the mechanisms involved are unclear.

Non-canonical WNT signalling might also affect neoangiogenesis. In a rat model of ischaemic myocardial injury, peri-infarct injection of conditioned medium from WNT11-overexpressing mesenchymal stem cells improved cardiac function and reduced infarct size, which was shown to involve non-canonical JNK–PKC signalling[73]. By contrast, mutations in *Wnt5a* or *Wnt11* in myeloid cells were associated with increased angiogenesis in mouse retinas mediated by downstream suppression of *Flt1* expression[74], which encodes an inhibitor of vascular endothelial growth factor (VEGF). These findings suggest potentially complex roles for non-canonical WNT signalling in the regulation of angiogenesis.

Inflammation

Extensive evidence links non-canonical WNT signalling to inflammation and inflammatory conditions such as rheumatoid arthritis[23,75], psoriasis[75–77] and atherosclerosis[23]. Indeed, non-canonical WNT signalling influences key mechanisms of vascular inflammation via multiple effects on vascular wall cells, thereby contributing to atherosclerosis[23].

WNT5A (but not WNT3A, a canonical WNT ligand) was shown to increase the expression of genes encoding pro-inflammatory factors, including cyclooxygenase 2, IL-6, IL-1α, Toll-like receptor 4, granulocyte colony-stimulating factor, granulocyte–macrophage colony-stimulating factor (GM-CSF), CC-chemokine ligand 2 (CCL2) and CCL8, in human aortic endothelial cells in vitro via non-canonical Ca^{2+}–PKC signalling[22]. These expression changes were associated with increased permeability of the endothelial cell monolayer[22]. WNT5A also activates pro-inflammatory nuclear factor-κB (NF-κB) signalling in endothelial cells[22,36]. Interestingly, activation of the Ca^{2+}–NFAT pathway, a downstream target of non-canonical WNT signalling, has been shown to induce a pro-inflammatory phenotype in human coronary artery endothelial cells in vitro that is characterized by an increased expression of pro-inflammatory molecules[78]. However, a direct link between non-canonical WNT signalling and NFAT signalling in endothelial cells has not yet been documented. By contrast, cardiac-specific overexpression of WNT11 in mice attenuated the inflammatory response to myocardial infarction and facilitated recovery after myocardial infarction in vivo[79]. This finding suggests an anti-inflammatory role for non-canonical WNT signalling in this context.

WNT5A can be secreted by circulating monocytes[59] and is involved in innate responses in monocytes and macrophages as evidenced by the upregulation of *WNT5A* gene expression in pathogen-activated macrophages and during monocyte differentiation into macrophages in response to GM-CSF and IL-4 treatment in vitro[80–82]. Importantly, pro-inflammatory and pro-oxidant signals, such as oxidized LDL, which are present in atherosclerotic plaques, upregulate the production of macrophage-derived WNT5A[83]. WNT5A in turn stimulates the secretion of pro-inflammatory cytokines (such as IL-1β, IL-6 and IL-8) from macrophages via Ca^{2+}–CaMKII signalling and facilitates foam cell formation[21,84,85]. Evidence suggests that WNT5A promotes transforming growth factor-β (TGFβ)-mediated macrophage polarization, as demonstrated in the context of kidney fibrosis[86]. Extensive research in oncology suggests that WNT5A has a wide range of effects on macrophage activation such as in establishing an NF-κB autocrine loop[87,88]. However, these WNT5A effects have not been fully investigated in atherosclerosis.

Several observational and mechanistic studies in in vivo models of atherosclerosis have further supported a role for non-canonical WNT signalling in promoting a vascular pro-inflammatory phenotype. In an *Apoe*$^{-/-}$ mouse model of atherosclerosis, *Wnt5a* knockdown inhibited lipid accumulation and inflammation in atherosclerotic plaques, which was suggested to involve ROR2 non-canonical WNT signalling and downstream nuclear translocation of NF-κB[89]. Furthermore, circulating WNT5A levels are increased in a variety of inflammatory processes, such as sepsis, as shown in experimental models, further supporting a connection between non-canonical WNT signalling and inflammation[23,75]. Despite the available strong evidence, further research is required to elucidate the full spectrum of the effects of non-canonical WNT ligands on cytokine production, inflammatory cell recruitment and activation, and overall lipid-driven and cytokine-driven vascular inflammatory responses.

Cellular insulin resistance

Observational data show that increased bioavailability of WNT5A in the circulation and high WNT5A expression in adipose tissue are both associated with systemic insulin resistance (defined as glucose intolerance) as first demonstrated by a team led by Walsh, who were pioneers in the study of the metabolic and cardiovascular implications of non-canonical WNT signalling[44,90–92]. This association could be indirectly linked to atherosclerosis via the detrimental effects of hyperglycaemia on vascular function[6]. However, evidence suggests that WNT5A is associated with molecular insulin resistance in the vasculature, defined as abnormal vascular insulin signalling[34,53]. Indeed, ex vivo insulin-mediated vasorelaxation of arterioles from visceral adipose tissue isolated from individuals with obesity was impaired compared with that of arterioles from subcutaneous adipose tissue from the same individuals[34]. JNK activation and *Wnt5a* expression was increased in visceral adipose tissue compared with subcutaneous fat[34]. Furthermore, in endothelial cells isolated from adipose tissue, treatment with recombinant WNT5A stimulated JNK activation and induced insulin resistance, demonstrated by a reduction in downstream AKT and eNOS phosphorylation[34]. Consistently, the capacity of WNT5A to abolish insulin-induced AKT and eNOS phosphorylation and NO production has been replicated in studies using primary endothelial cells from patients with diabetes[53].

Given the well-described link between JNK activity and molecular insulin resistance[93], the link between non-canonical WNT signalling, particularly the PCP

pathway, and induction of vascular insulin resistance is not surprising. Inflammatory factors, such as NF-κB and tumour necrosis factor, which are induced by non-canonical WNT signalling, can also interfere with molecular insulin signalling[94–97], providing indirect links between non-canonical WNT signalling and vascular insulin resistance. Finally, Ca^{2+}–PKC non-canonical WNT signalling might also contribute to vascular insulin resistance given that certain PKC isoforms directly induce cellular insulin resistance[6].

Adipose tissue secretome

Adipose tissue is a dynamic organ that interacts with the vascular wall in paracrine and endocrine manners via the secretion of biologically active molecules[5,98,99]. Metabolic diseases, such as visceral obesity and diabetes, are associated with a pro-inflammatory phenotype of visceral adipose tissue, which secretes molecules that can directly reach the vascular wall via the bloodstream and exert biological effects[100,101]. Epicardial adipose tissue is a multifaceted, dynamic marker of cardiometabolic disease that is affected by pleiotropic pharmacological therapies and influences the heart in multiple paracrine ways[98,102–105]. Perivascular adipose tissue (PVAT) can exert paracrine effects on the vasculature owing to its close proximity to the vascular wall and can also act as a receiver of biological signals from the vascular wall, thereby establishing bidirectional crosstalk with the vasculature[106]. PVAT senses signals of adjacent coronary inflammation, changing its phenotype and lipid content[107]. This phenomenon can be captured by an imaging biomarker of coronary inflammation derived from coronary CT angiography, which has been standardized and is used in clinical practice[107–110].

Our group has shown that WNT5A is the predominant WNT ligand expressed by PVAT surrounding internal mammary arteries in patients undergoing coronary artery bypass graft surgery[15]. WNT5A expression in PVAT is upregulated in the context of obesity and is independently associated with the activity of NADPH oxidases in the underlying vessels[15]. Furthermore, human primary VSMCs co-cultured with human adipocytes with *WNT5A* knockdown produced less NADPH oxidase-derived superoxide than VSMCs co-cultured with control adipocytes[15]. This finding confirms the concept that adipocyte-derived WNT5A might have paracrine pro-oxidant effects on vascular cells in humans. These findings are in agreement with a study reporting elevated levels of WNT5A and reduced levels of SFRP5 in the plasma of patients with peripheral occlusive arterial disease than in the plasma of healthy individuals[111]. Therefore, non-canonical WNT signalling, based on findings from the paradigm ligand WNT5A, is a novel paracrine link between obesity and vascular disease pathogenesis (FIG. 3).

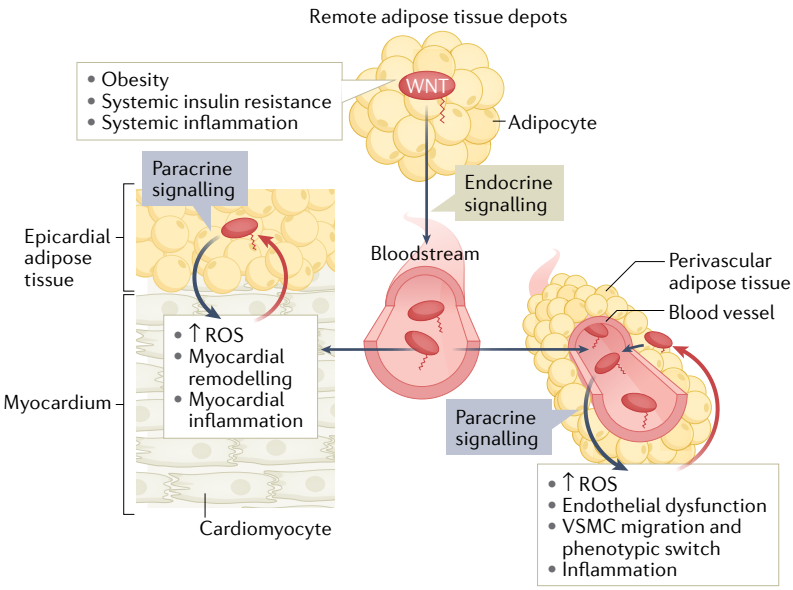

Fig. 3 | **Non-canonical WNT signalling and bidirectional interactions between adipose tissue depots and the cardiovascular system.** Obesity, systemic insulin resistance and systemic inflammation induce systemic upregulation of non-canonical WNT ligands, partly via upregulation of WNT secretion from adipose tissue depots. WNT ligands can reach the heart and blood vessels via the systemic circulation and from adjacent adipose tissue depots such as epicardial adipose tissue and perivascular adipose tissue, respectively. Regardless of the source, WNT ligands exert multiple effects on the cardiovascular system, including the induction of reactive oxygen species (ROS), myocardial remodelling, endothelial dysfunction, vascular smooth muscle cell (VSMC) migration and phenotypic switch, and inflammation. These changes in turn stimulate the release of signals that influence WNT expression (red arrows) in epicardial adipose tissue and perivascular adipose tissue, thereby establishing potential paracrine interaction loops between the cardiovascular system and adipose tissues.

Obesity and diabetes mellitus

Non-canonical WNT signalling has multiple broad roles related to the pathogenesis of metabolic diseases such as obesity and diabetes. These roles include multifaceted effects on adipose tissue, liver and pancreas, ranging from the regulation of energy storage and handling to adipogenesis and apoptosis[91,112,113]. Work by Fuster, Walsh and colleagues strongly suggests a causal association between obesity and increased bioavailability of WNT5A in the circulation and visceral adipose tissue in animal models and humans[15,90,91,114]. Furthermore, WNT5A overexpression in mouse myeloid cells augmented adipose tissue inflammation in vitro, and WNT5A directly induced JNK signalling and molecular insulin resistance in adipocytes of visceral adipose tissue in obese mice[91]. All these effects might influence obesity-related adipose tissue function and, indirectly, cardiovascular biology via regulation of the adipose tissue secretome.

Non-canonical WNT signalling influences adipose tissue biology in the context of obesity and diabetes in terms of adipose tissue volume, distribution, and secretome and, therefore, also influences the interactions between adipose tissue and the cardiovascular system. Non-canonical WNT signalling, mediated by WNT10B and WNT5A, is believed to have an anti-adipogenic effect via FZD and LRP receptors leading to reduced adipocyte size and also regulates the adipose tissue secretome and overall insulin sensitivity[115]. Non-canonical WNT signalling also induces adipose tissue inflammation independently of adipose tissue expansion[91]. In summary, non-canonical WNT signalling can contribute to the formation of small adipocytes, with low lipid content and increased inflammation, which are all hallmarks of visceral adipose tissue.

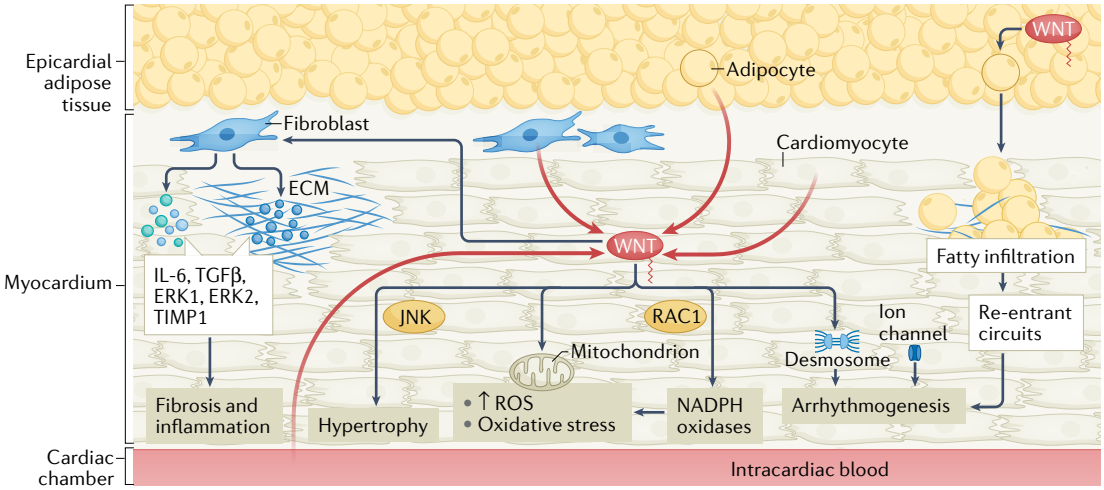

Fig. 4 | **Non-canonical WNT signalling in myocardial disease.** Summary of established and putative mechanisms linking non-canonical WNT signalling and myocardial disease. In the myocardium, sources of non-canonical WNT ligands, such as WNT5A, include cardiomyocytes, the microcirculation, blood in the cardiac cavities and the adjacent epicardial adipose tissue (red arrows). Non-canonical WNT signalling can stimulate cardiac fibroblasts, inducing the upregulation of expression of IL-6, transforming growth factor-β (TGFβ), ERK1 and ERK2, and tissue inhibitor of metalloproteinase 1 (TIMP1), thereby potentially promoting fibrosis and inflammation. Non-canonical WNT signalling can induce cardiac hypertrophy via JUN N-terminal kinase (JNK) activation. Non-canonical WNT signalling can promote oxidative stress through activation of NADPH oxidases mediated by the small GTPase RAC1. WNT might be linked to mitochondrial biology, regulating mitochondrial aggregation and the production of mitochondrial reactive oxygen species (ROS), although the direction of this interaction is unclear. WNT can also regulate desmosome and ion channel function, potentially promoting arrhythmogenesis. WNT secreted by epicardial adipose tissue can cause adipose tissue expansion and myocardial fatty infiltration, facilitating the development of re-entrant circuits and arrhythmias such as atrial fibrillation. ECM, extracellular matrix.

We must note that WNT ligands create a complicated, cross-interacting canvas in adipose tissue, which makes deciphering the integrated effects extremely challenging and prone to inaccuracies[115].

Non-canonical WNT signalling in cardiac diseases

Cardiac diseases, such as heart failure and arrhythmias, are caused by various pathogenic mechanisms such as arrhythmogenesis[116], dysregulated cardiac biomechanics[117], structural remodelling[118], abnormal energetics[119,120] and oxidative stress[121]. Observational evidence suggests that non-canonical WNT signalling is linked to cardiac diseases. For example, circulating levels of WNT5A were elevated in patients with heart failure compared with individuals without heart failure and were associated with haemodynamic markers of heart failure such as ejection fraction and filling pressures[122]. In a cohort of patients with dilated cardiomyopathy, higher plasma WNT5A levels were associated with increased right ventricular filling pressures and decreased right ventricular ejection fraction, and WNT5A expression was elevated in the right ventricle compared with the left ventricle[123]. Importantly, several experimental studies suggest that non-canonical WNT signalling is a causal regulator of cardiac disease (FIG. 4).

Arrhythmogenesis

Arrhythmias are caused by abnormal electrical impulse generation or conduction[116,124]. Abnormal impulses arise from increased automaticity, early afterdepolarizations causing slow action potential repolarization, or late diastolic depolarizations caused by sarcoplasmic reticulum Ca^{2+} leakage[124]. Abnormal conduction includes accessory pathways and re-entry circuits (often caused by a structural substrate such as regional fibrosis)[124]. Triggers include myocardial ischaemia, electrolyte disorders, medications and genetic channelopathies[116].

A large number of experimental studies have linked canonical WNT signalling to arrhythmogenic conditions such as arrhythmogenic cardiomyopathy, which is not surprising given the crucial role of β-catenin in the regulation of desmosomal intercellular junctions[125]. Furthermore, canonical WNT signalling regulates myocardial fibrosis, as shown in mouse models, thereby interfering with the formation of re-entry substrates such as in atrial fibrillation[126,127]. Both canonical β-catenin WNT signalling and non-canonical RHO-mediated WNT signalling have been associated with the pathophysiology of arrhythmogenic right ventricular cardiomyopathy (ARVC) in in silico models, which showed that the inactivation of the aforementioned pathways is linked with increased PPARγ expression and ARVC pathogenesis[128].

By contrast, the mechanistic role of non-canonical WNT signalling in human arrhythmogenesis has not been adequately explored. In a study in patients with rheumatic valve disease undergoing valve surgery, the presence of atrial fibrillation was associated with elevated expression of the transcription factor SNAIL1 and several WNT ligands, including the non-canonical WNT ligands WNT5A and WNT11, in the right atrium[129]. WNT5A has been associated with the upregulation of SNAIL1 protein levels in melanoma cells via

non-canonical PKC activation, which was associated with epithelial-to-mesenchymal transition (EMT) and metastasis potential[130]. Furthermore, WNT5A has been linked to TGFβ-dependent fibrosis and EMT in a variety of organs such as the liver[126,131]. Atrial fibrillation is tightly linked to processes such as EMT and fibrosis[132]. Therefore, these findings might imply a causal role for non-canonical WNT signalling in arrhythmogenesis by facilitating the development of re-entrant circuits.

Cardiac remodelling

Cardiac remodelling involves structural changes caused by myocardial wall stress (as a result of, for example, hypertension or valvular disease) and inflammation (such as after ischaemic myocardial injury)[118]. At the cellular level, remodelling is caused by cardiomyocyte hypertrophy and collagen deposition in the extracellular matrix caused by pro-inflammatory and redox signalling and metabolic signals[118,121,133]. These changes can induce arrhythmogenic substrates or impair contractile efficiency[120].

Non-canonical WNT signalling might contribute to cardiac fibrosis. For example, WNT5A was shown to stimulate human primary cardiac fibroblasts in vitro, inducing the production of IL-6 and tissue inhibitor of metalloproteinase 1 (TIMP1) and the activation of ERK1/ERK2 signalling[122]. This finding suggests that WNT5A can potentially promote inflammation and fibrosis in vivo. WNT5A also induces a reduction in glycogen synthase kinase 3β (GSK3β) levels in human cardiac fibroblasts in vitro, which promoted fibrosis partly via transactivation of TGFβ, although this effect seemed to be predominantly mediated by canonical WNT signalling[134]. In human ventricular cardiomyocytes, activation of non-canonical, Ca^{2+}-dependent WNT signalling stimulates the activation of the NFAT–calcineurin pathway[123], which has been shown to contribute to cardiac fibrosis in experimental models.

Non-canonical WNT signalling has been shown to stimulate cardiomyocyte hypertrophy[126]. More specifically, WNT5A activated PCP signalling mediated by Dapper 1 and led to downstream activation of JNK to promote hypertrophy in human cardiomyocytes in vitro, as assessed by microscopy and cardiomyocyte surface area[135]. Interestingly, in a mouse model of left ventricular hypertrophy induced by aortic constriction, both neutrophil depletion and myeloid-specific knockdown of *Wnt5a* reduced neutrophil infiltration in the myocardium and cardiac hypertrophy[136]. This finding suggests that WNT5A might also regulate cardiac hypertrophy in indirect ways involving neutrophils and local inflammatory responses in the heart.

Fatty infiltration of the myocardium is found in a spectrum of myocardial disease phenotypes ranging from arrhythmogenesis to contractile dysfunction[137]. Although suppression of canonical WNT signalling has been linked to fatty infiltration of the myocardium, for example, in animal models of ARVC[138], limited evidence exists on non-canonical WNT signalling and fatty remodelling of the myocardium. Non-canonical WNT signalling has been implicated in fatty infiltration in other organs[139,140] such as in a mouse model of non-alcoholic fatty liver disease, which was rescued by canonical WNT3A signalling[141].

Myocardial energetics

Under physiological conditions, fatty acid oxidation is the major source of ATP in the heart, followed by glycolysis and lactate metabolism[120]. Different myocardial diseases are associated with different metabolic profiles but most eventually lead to ATP depletion, reducing contractile efficiency and altering a wide range of downstream signalling events[142]. Several types of myocardial dysfunction are characterized by a shift towards glycolysis and ketone body oxidation to meet the increasing energy demands of the failing heart under stress[143]. Medications such as sodium–glucose cotransporter 2 inhibitors might exert some of their beneficial effects by regulating ketone body metabolism, glycolysis substrate inflow and fatty acid oxidation[144], which highlights the concept that metabolic pathway balance and fuel shifts might be crucial for modifying myocardial performance[143].

Evidence suggests that WNT signalling mediates metabolic pathway reprogramming in a variety of cell types although this action mainly involves canonical signalling[145,146]. Non-canonical WNT signalling attenuates mitochondrial aggregation in the HEK93 cell line[147] and protects mitochondria from fission–fusion alterations and prevents mitochondrial loss in neurons[148], both via PKC and regulation of intracellular Ca^{2+} dynamics. Overexpression of the non-canonical WNT ligand WNT11 preserves mitochondrial membrane potential and protects cardiomyocytes against hypoxia in vitro via mechanisms involving insulin-like growth factor 1 and VEGF[149]. Non-canonical WNT signalling also accelerates glucose oxidative metabolism in the liver, which leads to steatosis via de novo lipogenesis[150]. Although these findings provide putative links to myocardial metabolism, the role of non-canonical WNT signalling in myocardial energetics has not been directly explored.

Oxidative stress

Oxidative stress has a key pathophysiological role in myocardial diseases such as in ischaemia–reperfusion injury after myocardial infarction[151] and through redox signalling in heart failure and arrhythmia[152]. Oxidative stress results from ischaemia–reperfusion injury[153], inflammation-mediated stimulation of NADPH oxidases[121,154] and disturbed mitochondrial energetics[120,155]. Myocardial ROS, in turn, can induce hypertrophy, apoptosis, autophagy, metabolic enzyme dysregulation and impaired contractility, thereby drastically contributing to myocardial disease[11,121,153,155].

Ischaemia–reperfusion injury is characterized by sudden oxygen abundance in a stunned myocardium, in which free radicals are produced because of an imbalance between pro-oxidant and antioxidant enzymes[151,153]. The excess free radicals induces cardiomyocyte death via multiple intracellular pathways, including proteolysis, caspase activation and mitochondrial regulation[151,153]. Co-expression of AKT1 and the non-canonical ligand WNT11 stimulates the proliferation and differentiation of mesenchymal stem cells into cardiomyocytes and attenuates hypoxia–reperfusion injury[156]. By contrast, a study in mice showed that blockade of non-canonical WNT signalling attenuates myocardial ischaemia–reperfusion injury[157]. NADPH oxidases have been implicated in atrial

fibrillation and heart failure via a multitude of redox-sensitive intracellular transcription pathways[154,158]. Non-canonical WNT signalling is linked to NADPH oxidases via RAC1, as shown in VSMCs in vitro[15]. Whether these findings are relevant to the human myocardium remains to be proven.

Targeting non-canonical WNT signalling

The studies discussed above indicate a strong mechanistic link between non-canonical WNT signalling and cardiovascular disease. Therefore, successful targeting of this pathway could have multiple beneficial effects in patients, especially in the context of obesity, in which the bioavailability of the principal non-canonical WNT ligand, WNT5A, is increased[15,23]. Interestingly, the downstream mediators of non-canonical WNT signalling (PLC–CaMKII, RAC1, RHOA and JNK) are convergence points for multiple non-WNT pathways such as the catecholamine and renin–angiotensin–aldosterone pathways[159–161]. The exact contribution of WNT ligands to cardiovascular pathophysiology in this context is unknown. However, targeting WNT signalling might offer an advantage over targeting downstream molecules, which would also influence the outputs of multiple other, non-WNT pathways.

Non-canonical WNT signalling is a conserved pathway affecting virtually all cell types and governing fundamental biological processes[18,162]. Consequently, its targeting is prone to non-specific, off-target adverse effects (with tumorigenesis being the most important) and is challenging when embryonic development is relevant (for example, in pregnant women)[18,162]. The interconnected network of WNT ligands and receptors further complicates targeting a single ligand[18,162].

So far, early attempts have mainly focused on targeting the canonical WNT signalling pathway, especially in the context of cancer[18,162,163]. By contrast, targeting of non-canonical WNT signalling has not been explored. Advances in biotechnology, gene editing and drug delivery, coupled with a better understanding of the downstream mediators of non-canonical WNT signalling, might help towards this end.

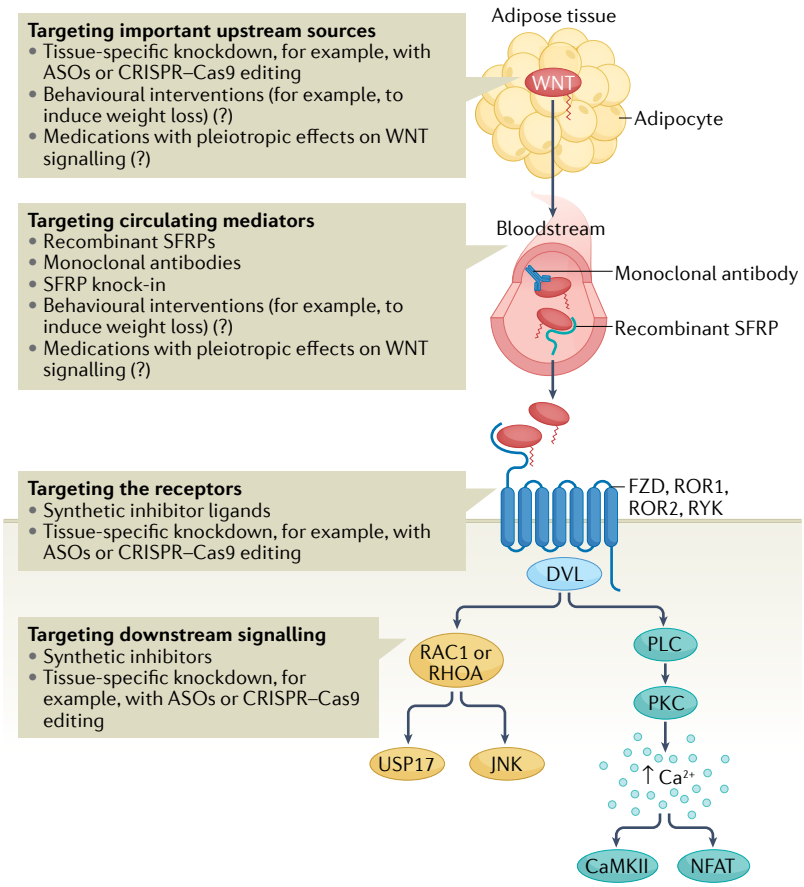

Fig. 5 | **Potential strategies for therapeutic targeting of non-canonical WNT signalling.** Modulating WNT effects can be achieved by targeting the main sources of WNT ligands, such as adipose tissue, with the use of tissue-specific knockdown technologies, for example, antisense oligonucleotides (ASOs) and CRISPR–Cas9 methods. However, these approaches require considerable research before their eventual translation into the clinic. The balance between different circulating WNT ligands can be targeted by using recombinant decoy receptors of WNT ligands (such as secreted frizzled-related proteins (SFRPs)) or monoclonal antibodies against WNT ligands. Overall, targeting of WNT signalling systemically might be associated with off-target adverse effects. WNT signalling can also be targeted by blocking or decreasing WNT receptors, with the added theoretical benefit of tissue specificity when using knockdown methods. Finally, downstream signalling could be targeted by developing chemical inhibitors or knockdown technologies. Targeting individual downstream molecules instead of the WNT ligands would provide a theoretical benefit of more specifically targeting individual subphenotypes caused by WNT signalling activation. Given that obesity is associated with increased WNT bioavailability, behavioural interventions, such as interventions to induce weight loss, and the use of medications with known metabolic effects might have pleiotropic effects on WNT signalling although the effect of these approaches on WNT signalling is unclear. CaMKII, Calcium/calmodulin-dependent protein kinase II; DVL, Dishevelled; FZD, Frizzled; JNK, JUN N-terminal kinase; NFAT, nuclear factor of activated T cells; PKC, protein kinase C; ROR, tyrosine-protein kinase transmembrane receptor ROR; RYK, tyrosine-protein kinase RYK.

Targeting strategies

Targeting non-canonical WNT signalling might be theoretically achieved with the use of chemical inhibitors of, for example, SFRPs or RAC1 (REFS[15,18]). The use of monoclonal antibodies, antisense oligonucleotides (ASOs) and gene-editing methods (such as CRISPR–Cas9) can allow more efficient targeting of non-canonical WNT signalling than previously thought[164–167]. More efficient drug delivery to target tissues might also be achieved with the use of nanoparticles[164,168,169]. The main challenge of using monoclonal antibodies is their systemic, whole-body delivery, posing the risk of substantial adverse effects resulting from systemic WNT inhibition in tissues outside of the cardiovascular system. This issue might be bypassed by using tissue-specific targeting of WNT elements with tools such as ASOs and CRISPR–Cas9 editing. However, these strategies are not clinically feasible at present and require extensive validation and optimization. Nanoparticle technology might be used to guide drugs and vectors to sites of interest via systemic or local administration of nanoparticles with affinity to particular molecules such as endothelial markers (for example, vascular cell adhesion molecules). This approach, in combination with molecular techniques (CRISPR–Cas9 or ASOs), could increase local efficacy and tissue specificity of WNT targeting, as demonstrated with the use of macrophage-specific, promoter-driven plasmids contained in lipid nanoparticles to target inflammatory cells such as macrophages[170]. The characteristics of these strategies are summarized in FIG. 5.

Potential targets

Non-canonical WNT signalling comprises a complex network of receptors and downstream molecules. Therefore, one could, in theory, interfere with non-canonical WNT signalling at different levels by using the techniques mentioned in the previous section. Importantly, the canonical and non-canonical pathways cross-regulate each other; therefore, any intervention on non-canonical WNT signalling is expected to also have effects on canonical WNT signalling[162,171].

WNT ligands and SFRP inhibitors. Targeting non-canonical WNT ligands would be the most obvious way to inhibit all downstream signalling. However, simultaneous targeting of all non-canonical WNT ligands would not be feasible. WNT5A is a paradigm non-canonical WNT ligand, having been the focus of most research on non-canonical WNT signalling; therefore, WNT5A would seem to be an appropriate therapeutic target in this context[15,63]. Systemic WNT5A targeting (for example, with monoclonal antibodies or exogenously administered recombinant forms of SFRPs) might be the most obvious way forward, but this approach would pose the aforementioned risks of global inhibition. Considering the pathophysiological mechanisms evaluated in the previous sections, tissue-specific (for example, adipose tissue-specific) targeting would be more efficient, although still not feasible at present.

WNT receptors. Downregulation of WNT receptors, such as FZD2 and FZD5, could markedly attenuate non-canonical WNT signalling. This strategy could be important given that FZD2 and FZD5 have been shown to be upregulated in internal mammary arteries from individuals with obesity, suggesting a higher sensitivity to WNT ligands[15]. Downregulation of WNT receptors could be achieved by ASO-mediated silencing or CRISPR–Cas9 gene editing, in which the use of tissue-specific promoters would be ideal. However, as mentioned, these strategies remain hypothetical at present.

Downstream signal transduction molecules. Non-canonical WNT signalling converges on a number of downstream molecules, including RAC1, JNK, CaMKII and PKC as well as USP17, a newly described WNT5A target[15,52]. Targeting of these downstream targets might reverse the detrimental effects of non-canonical WNT signalling. However, many non-WNT pathways also converge on these downstream molecules, which would make their targeting prone to non-specific effects.

Studies in mouse models of RAC1 depletion have reported beneficial effects, such as reduced endoplasmic reticulum stress and reduced cardiac oxidative stress[172]. In addition, several allosteric inhibitors of RAC1 have been developed. Several JNK inhibitors have been used in animal models of diseases such as neurodegeneration[173] and in a phase Ib clinical trial in patients with pulmonary fibrosis[174], supporting the clinical relevance of targeting JNK. Similarly, CaMKII inhibition in vivo is associated with decreased atherosclerotic plaque burden in *Apoe*$^{-/-}$ mice[175]. Administration of the CaMKII inhibitor KN93 in a mouse model of heart failure induced by pressure overload had beneficial effects[176]. PKC inhibition in vivo is more challenging given the large number of PKC isoforms[6]. Finally, USP17 is a newly identified link between WNT signalling, RAC1 activation and downstream redox signalling, which might warrant further investigation as a potential therapeutic target[15].

Conclusions

Non-canonical WNT signalling, acting via receptor-mediated pathways and through activation of second messengers, such as RAC1, JNK, Ca^{2+}-mediated CaMKII and PKC, is causally linked to vascular and myocardial disease in animal and human experimental models. In particular, non-canonical WNT signalling induces vascular oxidative stress via NADPH oxidase activation, promotes endothelial dysfunction and insulin resistance via JNK signalling and oxidative eNOS uncoupling, increases vascular inflammation in endothelial cells and macrophages, and triggers a VSMC contractile-to-synthetic phenotypic switch that might promote atherosclerotic plaque instability. Importantly, non-canonical WNT signalling is upregulated in adipose tissue (including PVAT) in obesity, exerting paracrine and endocrine vascular effects. Non-canonical WNT signalling has putative links to a wide spectrum of cardiac disease phenotypes via regulation of myocardial metabolism, fibrosis, and adipogenesis pathways and of redox signalling mediators such as NADPH oxidases and NF-κB, potentially contributing to myocardial remodelling, arrhythmogenic substrate formation and contractile dysfunction.

Non-canonical WNT signalling is therefore a multifaceted causal mediator of atherosclerosis and a link between obesity and vascular disease. Advances in biotechnology have opened up new potential approaches to modulate non-canonical WNT signalling through targeting specific tissues, proteins or genes related to WNT ligands, receptors or the downstream signalling network, all of which warrant further investigation.

Published online 13 June 2022

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Acknowledgements
C.A. acknowledges support from the British Heart Foundation (CH/F/21/90009, TG/19/2/34831 and RG/F/21/110040), the Oxford BHF Centre of Research Excellence (RE/18/3/34214) and the Oxford NIHR Biomedical Research Centre.

Author contributions
I.A. and M.P. researched data for the article. I.A. and C.A. contributed to the discussion of content, and I.A. wrote the manuscript. All the authors reviewed and/or edited the manuscript before submission.

Competing interests
C.A. is founder, shareholder and director of Caristo Diagnostics, a CT image analysis company. The other authors declare no competing interests.

Peer review information
Nature Reviews Cardiology thanks the anonymous reviewer(s) for their contribution to the peer review of this work.

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