Perivascular adipose tissue-derived Wnt5a as a regulator of human vascular disease pathogenesis

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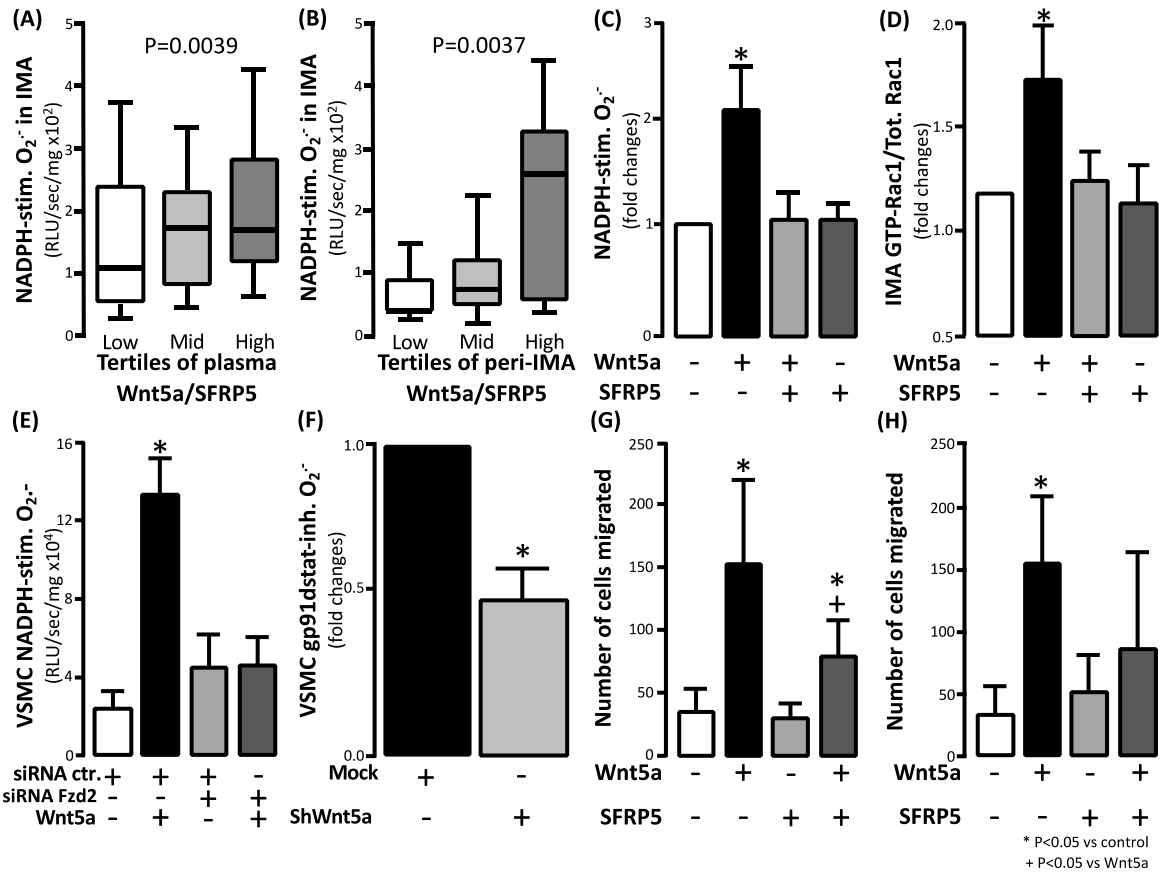
Background: Wnt5a exerts its biological roles via receptor-mediated non-canonical Wnt signalling, and its systemic levels are upregulated in obesity. However, the vascular effects of Wnt5a are poorly explored.

Purpose: We hypothesised that Wnt5a derived from perivascular adipose tissue (PVAT) triggers vascular disease by controlling redox signalling in the human vascular wall, effects that could be regulated by its decoy receptor, Sfrp5.

Methods: The study included 1,003 patients undergoing cardiac surgery. Fasting plasma, internal mammary artery (IMA) and peri-IMA PVAT samples were collected during surgery and were used for gene expression studies and functional assays. Primary VSMC were isolated from vascular segments and used for *in vitro* experiments. Superoxide ($O_2^{\cdot-}$) generation was measured by lucigenin (5μM) chemiluminescence with NADPH 100μM stimulation used as indicator of NADPH-oxidases activity. Activation of Rac1, a key NADPH-oxidases subunit, was evaluated by a commercially available kit. VSMC migration was evaluated by Boyden's chamber. Gene expression studies were performed by TaqMan probes. Circulating Wnt5a and Sfrp5 were measured by ELISA.

Results: The circulating Wnt5a/Sfrp5 ratio was positively associated with increased NADPH-oxidases activity in human IMA (A). Furthermore, increased Wnt5a/Sfrp5 expression ratio in PVAT was also associated with elevated NADPH-oxidases activity in the adjacent IMA (B). *Ex vivo* 45min incubation of IMA segments with recombinant human Wnt5a 100ng/mL resulted in direct stimulation of NADPH-oxidases (C) and GTP-activation of its associated subunit Rac1 (D). These effects were abolished in the presence of Sfrp5 300ng/mL. SiRNA knockdown of frizzled 2 (Fzd2, a Wnt receptor) abolished the ability of recombinant Wnt5a to stimulate NADPH-oxidases activity in human VSMC (E). Co-culture of VSMC with Wnt5a-KO primary adipocytes resulted in reduced gp91 dstat-inhibitable $O_2^{\cdot-}$ generation, suggesting that Wnt5a secreted by AT may stimulate the NOX2 isoform of NADPH-oxidases (F). Finally, recombinant Wnt5a 100ng/mL stimulated VSMC migration which was attenuated by Sfrp5 (G) and pegylated superoxide dismutase (peg-SOD) 300U/mL (H), suggesting that this effect is redox sensitive.

Conclusions: We demonstrate for the first time that Wnt5a from both the systemic circulation and PVAT, induces NADPH-oxidases activity in human arteries presumably via Fzd2-mediated activation of non-canonical Wnt signalling that involves Rac1, resulting in VSMC migration. Vascular oxidative stress and VSMC migration, in turn, are key steps in atherogenesis progression. As such, considering that Wnt5a and its vascular receptors are upregulated in obesity, this molecule may be a novel therapeutic target for the prevention and treatment of vascular complications in obesity and diabetes.


(A)
P=0.0039
NADPH-stim. O2- in IMA
(RLU/sec/mg x10²)
Low
Mid
High
Tertiles of plasma
Wnt5a/SFRP5
(B)
P=0.0037
NADPH-stim. O2- in IMA
(RLU/sec/mg x10²)
Low
Mid
High
Tertiles of peri-IMA
Wnt5a/SFRP5
(C)
NADPH-stim. O2-
(fold changes)
Wnt5a - + + -
SFRP5 - - + +
(D)
IMA GTP-Rac1/Tot. Rac1
(fold changes)
Wnt5a - + + -
SFRP5 - - + +
(E)
VSMC NADPH-stim. O2.-
(RLU/sec/mg x10⁴)
siRNA ctr. + + + -
siRNA Fzd2 - - + +
Wnt5a - + - +
(F)
VSMC gp91dstat-inh. O2-
(fold changes)
Mock + -
ShWnt5a - +
(G)
Number of cells migrated
Wnt5a - + - +
SFRP5 - - + +
(H)
Number of cells migrated
Wnt5a - + - +
SFRP5 - - + +
* P<0.05 vs control
+ P<0.05 vs Wnt5a