Abstract: P356

Fzd5 signalling controls vascular growth by repressing Ets1-mediated transcription of Angpt2 and Flt1

Authors:
MM Brandt¹, CGM Van Dijk², I Chrifi¹, HM Kooi³, L Louza-Martinez², J Pei², RJ Rottier³, MC Verhaar², DJ Duncker¹, C Cheng², ¹Erasmus Medical Center, Experimental Cardiology, Department of Cardiology - Rotterdam - Netherlands, ²University Medical Center Utrecht, Department of Nephrology and Hypertension, Division of Internal Medicine and Dermatology - Utrecht - Netherlands, ³Erasmus Medical Center, Department of Paediatric Surgery - Rotterdam - Netherlands,

Topic(s):
Microcirculation, Angiogenesis, Arteriogenesis

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S91

Funding Acknowledgements:
Netherlands Foundation for Cardiovascular Excellence [to C.C.], The Netherlands Organisation for Scientific Research Vidi grant [no. 91714302 to C.C.]

Background: Formation of a functional vascular system is a dynamic and highly regulated process initiated during embryogenesis, which continues to play important roles throughout life in both health and disease. Understanding the mechanisms involved in this process is essential for many areas of research, as this knowledge provides the foundation for therapeutic applications targeting disturbed angiogenesis (i.e. to limit tumour growth or plaque instability). In previous studies, Fzd5 was shown to be critically involved in neovessel formation, as Fzd5 knock-out mice showed a lethal deficiency in placenta and yolk sac angiogenesis. These studies thus indicate that Fzd5 signalling is indispensable for angiogenesis, but to date the exact molecular underpinning of these observations remains elusive. Here we investigated the mechanism by which signalling via this receptor occurs in endothelial cells (ECs) and how this affects angiogenesis.

Methods and results: Using short interference RNA mediated loss of function assays, the function and mechanism of signalling via Fzd5 was studied in primary human ECs. Our findings in a collagen matrix-based 3D co-culture of primary vascular cells indicate that endothelial Fzd5 signalling promotes neovessel formation, as knockdown of Fzd5 diminished angiogenesis by more than 60% (n=4, P<0.05). Silencing endothelial Fzd5 expression limited proliferation, as a result of G0/G1 cell cycle arrest (G0/G1: siNon-Target 30.4% siFzd5: 50.6%, n=3, P<0.05), and led to a five-fold decrease in cell migration capacity (n=4, P<0.05). Furthermore, Fzd5 knockdown resulted in a doubling of Angpt2 and Flt1 expression (n=11, P<0.05), factors that are mainly known for their destabilizing effects on the vasculature. Upregulation of Angpt2 and Flt1 in Fzd5 silenced ECs was induced by enhanced PKC signalling, as inhibition of PKC by Staurosporine (5-20nM) led to a dose-dependent suppression of both factors (n=5, P<0.05). Canonical Wnt signalling, non-canonical Wnt/Ca2+-mediated activation of NFAT, and non-canonical Wnt/PCP-mediated activation of JNK were not causally linked to the upregulation of Flt1 and Angpt2. We demonstrated that PKC-induced transcription of Angpt2 and Flt1 involved the transcription factor Ets1, as a combined knockdown of Fzd5 and Ets1 completely blocked the enhanced Angpt2 expression, and reduced the upregulation of Flt1 by almost 50% (both n=4, P<0.05). In addition, an intervention with knockdown of Ets1 on top of the Fzd5 knockdown also partially rescued the poor angiogenic phenotype observed in the 3D co-culture model (n=6, P<0.05), indicating that this transcription factor was critically involved in suppressing angiogenesis in absence of Fzd5.

Conclusions: The current study provides evidence for a pro-angiogenic role of Fzd5, which was shown to be involved in endothelial tubule formation, cell cycle progression and migration, and does so by repression of PKC/Ets1-mediated transcription of Flt1 and Angpt2.
Abstract:
Pzd5 signalling controls vascular growth by repressing Ets1-mediated transcription of Angpt2 and Flt1.

Authors:
MM Brandt1, CGM Van Dijk2, I Chrifi1, HM Kool3, L Louzao-Martinez2, J Pei2, RJ Rottier3, MC Verhaar2, DJ Duncker1, C Cheng2

1 Erasmus Medical Center, Experimental Cardiology, Department of Cardiology - Rotterdam - Netherlands,
2 University Medical Center Utrecht, Department of Nephrology and Hypertension, Division of Internal Medicine and Dermatology - Utrecht - Netherlands,
3 Erasmus Medical Center, Department of Paediatric Surgery - Rotterdam - Netherlands,

Topic(s):
Microcirculation, Angiogenesis, Arteriogenesis

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S91

Funding Acknowledgements:
Netherlands Foundation for Cardiovascular Excellence [to C.C.], The Netherlands Organisation for Scientific Research Vidi grant [no. 91714302 to C.C.]

Background: Formation of a functional vascular system is a dynamic and highly regulated process initiated during embryogenesis, which continues to play important roles throughout life in both health and disease. Understanding the mechanisms involved in this process is essential for many areas of research, as this knowledge provides the foundation for therapeutic applications targeting disturbed angiogenesis (i.e. to limit tumour growth or plaque instability). In previous studies, Fzd5 was shown to be critically involved in neovessel formation, as Fzd5 knockout mice showed a lethal deficiency in placenta and yolk sac angiogenesis. These studies thus indicate that Fzd5 signalling is indispensable for angiogenesis, but to date the exact molecular underpinning of these observations remains elusive. Here we investigated the mechanism by which signalling via this receptor occurs in endothelial cells (ECs) and how this affects angiogenesis.

Methods and results: Using short interference RNA mediated loss of function assays, the function and mechanism of signalling via Fzd5 was studied in primary human ECs. Our findings in a collagen matrix-based 3D co-culture of primary vascular cells indicate that endothelial Fzd5 signalling promotes neovessel formation, as knockdown of Fzd5 diminished angiogenesis by more than 60% (n=4, P<0.05). Silencing endothelial Fzd5 expression limited proliferation, as a result of G0/G1 cell cycle arrest (G0/G1: siNon-Target 30.4% siFzd5: 50.6%, n=3, P<0.05), and led to a five-fold decrease in cell migration capacity (n=4, P<0.05). Furthermore, Fzd5 knockdown resulted in a doubling of Angpt2 and Flt1 expression (n=11, P<0.05), factors that are mainly known for their destabilizing effects on the vasculature. Upregulation of Angpt2 and Flt1 in Fzd5 silenced ECs was induced by enhanced PKC signalling, as inhibition of PKC by Staurosporine (5–20nM) led to a dose-dependent suppression of both factors (n=5, P<0.05). Canonical Wnt signalling, non-canonical Wnt/Ca2+-mediated activation of NFAT, and non-canonical Wnt/PCP-mediated activation of JNK were not causally linked to the upregulation of Flt1 and Angpt2. We demonstrated that PKC-induced transcription of Angpt2 and Flt1 involved the transcription factor Ets1, as a combined knockdown of Fzd5 and Ets1 completely blocked the enhanced Angpt2 expression, and reduced the upregulation of Flt1 by almost 50% (both n=4, P<0.05). In addition, an intervention with knockdown of Ets1 on top of the Fzd5 knockdown also partially rescued the poor angiogenic phenotype observed in the 3D co-culture model (n=6, P<0.05), indicating that this transcription factor was critically involved in suppressing angiogenesis in absence of Fzd5.

Conclusions: The current study provides evidence for a pro-angiogenic role of Fzd5, which was shown to be involved in endothelial tubule formation, cell cycle progression and migration, and does so by repression of PKC/Ets1-mediated transcription of Flt1 and Angpt2.