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TGF-β family signaling in endothelial cells and angiogenesis

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Citation
Ma, J. (2021, September 30). *TGF-β family signaling in endothelial cells and angiogenesis*. Retrieved from https://hdl.handle.net/1887/3214214

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/3214214

Note: To cite this publication please use the final published version (if applicable).

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English summary

Transforming growth factor-β (TGF-β) family proteins are secreted factors with pleiotropic roles in cell-to-cell communication. A large number of studies have revealed their key function in a multitude of biological processes and activities. For endothelial cells (ECs), TGF-β family ligands, including TGF-β and bone morphogenetic proteins (BMPs), play important roles in regulating ECs behaviour and function, including cell proliferation, migration, sprouting and differentiation. Imbalance of TGF-β signaling in ECs due to (epi)genetic changes resulting in gain- or loss-of- protein function is often associated with vascular disease. The activation of TGF-β signaling in ECs can activate a phenotypic switch to mesenchymal-like cells, also termed endothelial-to-mesenchymal transition (EndMT). EndMT has broad functions in human health; it is indispensable for cardiovascular system formation in embryonic development, but it also contributes to some diseases, such as fibrosis and cancer. Thus, TGF-β is considered as a potential therapeutic target for EndMT-related diseases. Moreover, achieving a precise control of EndMT in vitro and in vivo provides new therapeutic opportunities for tissue regeneration. In **Chapter 2**, we summarized the mechanisms by which TGF-β signaling pathway mediates EndMT and the contribution of TGF-β-induced EndMT to the development of fibrotic diseases and cancer. We also summarized the potential applications of TGF-β-induced EndMT in tissue engineering and repair.

EndMT is intricately regulated by multiple transcription factors, including factors that promote or inhibit this process. Perturbations of the action of such factors may lead to pathological conditions. New putative factors that regulate this process continue to be identified, for which their physio-pathological significance needs to be verified. In that case, an efficient, reliable methodology to investigate EndMT is needed. In **Chapter 3**, we described a workflow to assess the role of cytokines, such as TGF-β family members, in EndMT. We approached this by investigating cell morphology changes and changes in the expression of endothelial and mesenchymal-specific markers. We made use of the CRISPR/CAS9 gene editing technology to specifically perform gene knockouts. This methodology and its application to study the role of a specific factor in triggering or regulating EndMT, is described.

TGF-β is known as a major inducer of EndMT, while the effects of BMPs on this process is unclear. In **Chapter 4**, we demonstrated that TGF-β2, but not BMP9, induced EndMT in murine endothelial MS-1 and 2H11 cells. Mechanistically, the expression of SNAIL and SLUG, which are pivotal effectors for TGF-β2-triggered EndMT, are upregulated by the activation of TGF-β signaling. We elucidated why BMP9, despite inducing SNAIL and SLUG expression, fails to trigger EndMT. We found that BMP9 strongly increases the expression of inhibitor of DNA binding (ID) proteins, which help ECs to maintain their original traits. ID proteins antagonize SNAIL and SLUG-inducing effects in EndMT. We therefore conclude that the balance between SNAIL and ID activity determines TGF-β-induced EndMT.

Cancer genetic changes drive cells to highly proliferate and extravasate into the systemic blood circulation to spread into other organs or tissues in the body. The tumor cells stimulate angiogenesis, not only to provide nutrients and oxygen for tumor growth, but also to support metastasis. The latter of which is the main reason for the death of cancer patients. Efficient, low-cost and time-saving in vivo models to investigate cancer progression are needed to develop new treatment options. In **Chapter 5**, we summarized the different xenograft models

in embryonic zebrafish to study breast cancer cell intravasation, extravasation and tumor angiogenesis. In particular we focused on the role of TGF-β therein.

Disturbances in EC behaviour can compromise angiogenesis. Mis-regulation of EC function contributes to the development of neovascularization related diseases, including cancer and ocular disorders. In **Chapter 6**, we synthesized and identified two novel small molecule macrocyclic BMPRI kinase inhibitors, named OD16 and OD29. Both compounds efficiently inhibit BMPRI mediated signaling, and OD29 also antagonizes VEGF-induced extracellular regulated kinase MAP kinase phosphorylation in ECs. OD16 and OD29 inhibit ECs functions in vitro, including cell migration, invasion and cord formation. The compounds also show inhibitory effects in normal and tumor angiogenesis in vivo. Taken together, our data suggests that they have potential as anti-angiogenesis therapeutic agents.

In summary, this thesis is focused on the understanding of the mechanisms underlying EndMT and EC-mediated angiogenesis. I expect that this thesis will contribute to the development of therapeutic options for the treatment of vascular diseases.