Understanding the recruitment process

How endothelial cells control pericyte proliferation, invasion and recruitment

Commentary on *'Defining endothelial cell-derived factors that promote pericyte recruitment and capillary network assembly'* Scott S. Kemp, Kalia N. Aguera, Byeong Cha, George E. Davis. 20 Aug 2020https://doi.org/10.1161/ATVBAHA.120.314948

Capillary vessel networks are carefully assembled and sustained via a series of complex, highly regulated processes which, if impaired, result in a range of pathological conditions, including tumor angiogenesis, diabetic microangiopathy, stroke and dementia[1]. Key to the maturation and maintenance of healthy vessels is the close association between endothelial cells (EC) and pericytes, which critically contribute to vessel stability and homeostasis through direct cell-cell interaction, paracrine influence and deposition of extracellular matrix (ECM) to the basement membrane[2]. The molecular pathways that control pericyte recruitment to developing capillary networks are only partially understood. For example, genetic disruption of EC-pericyte platelet-derived growth factor (PDGF)-BB – PDGFRβ signalling implicated an important role for this pathway in pericyte recruitment[3-5], but the incomplete abolition suggests a complex process co-ordinately orchestrated by multiple factors. Compensatory responses and early lethality are among the factors that confound *in vivo* studies to elucidate the principal factors that drive pericyte recruitment. While co-culture models have been employed to address these questions *in vitro*, they have typically assayed the close apposition of pericytes with primitive EC tubes, and fall short of assessing the direct functional growth factor requirements for pericyte invasion of collagen matrices, their proliferation and recruitment to complex EC networks.

In the current issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Kemp et al.[6] present a thorough re-evaluation of the EC-derived factors responsible for pericyte recruitment and vessel maturation, having established advanced culture systems that accurately model complex three-dimensional capillary network formation *in vitro*. Employing pericyte-only assays, the authors identified PDGF-BB, PDGF-DD, and endothelin-1 (ET-1) as primary stimulators of pericyte proliferation and invasion. While previously implicated factors, transforming growth factor (TGF)β and HB-EGF, were insufficient to promote invasion, HB-EGF promoted pericyte proliferation and a novel 'priming' role was ascribed to TGFβ. Treatment with TGFβ enhanced pericyte sensitivity to PDGF-BB, PDGF-DD, and ET-1, which resulted in a more vigorous invasion. With these insights, the authors sought to directly test roles for these pathways in pericyte recruitment to EC tubes. Their optimised co-culture model created highly interconnected three-dimensional networks, the dynamic assembly of which was captured over 72 hours in time-lapse movies. Importantly, only pericytes presenting with

elongated morphology on the EC tube were classed as 'recruited', distinguishing them from those migrating in close proximity. Basement membrane deposition provided a second functional readout for recruited pericytes. Kemp and colleagues confirmed the synergistic requirement for PDGF-BB, PDGF-DD, ET-1, TGFβ and HB-EGF to control pericyte-EC interaction and for complete establishment of capillary networks. A cocktail of pharmacological inhibitors targeting these pathways, dubbed CIGS, almost entirely abolished pericyte recruitment, a result which was further validated through use of neutralising antibodies against the EC-derived growth factors. These experiments defined PDGF-BB and ET-1 as the most potent cues for attracting pericytes and yet these factors, in combination, were insufficient to fully drive the process. Combined targeting of the five factors was required to reduce the number of elongated pericytes to <10% of control level. Failure to recruit sufficient pericytes impaired the processes of EC tube remodelling and maturation required to produce elongated, branched capillary networks.

Collectively, the findings of this study reinforce and extend our understanding of the reciprocal EC-pericyte interactions and paracrine control of capillary network formation[2] (**Figure**). A remaining challenge is to determine whether the pathways implicated are sufficient and necessary to promote pericyte recruitment *in vivo* or whether as yet unidentified factors are additionally required. Although the deposition of some ECM components was recapitulated in the improved co-culture model of Kemp, it is important to bear in mind that *in vivo* regulation may ostensibly differ, due to the local ECM-controlled signalling micro-environments[7], which may or may not be faithfully recapitulated *in vitro*. The finding that TGFβ primes pericyte responses to PDGFs and ET-1 is intriguing and further research is warranted to delineate the molecular mechanisms that mediate this phenomenon. Synergy between signalling pathways has been reported to enhance mural cell recruitment to tumor vasculature, with fibroblast growth factor (FGF)2-induced PDGF receptor expression in EC[8] and in pericytes[9] enhancing the sensitivity of those cells to PDGF-BB.

In conclusion, Kemp and colleagues provide novel insights into the processes underpinning EC-mural cell assembly and present a useful model system that can be applied to investigate mechanisms of vasculogenesis in the context of development and disease and for regenerative strategies, such as the creation of functional vascularised tissue grafts[10].

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Disclosure

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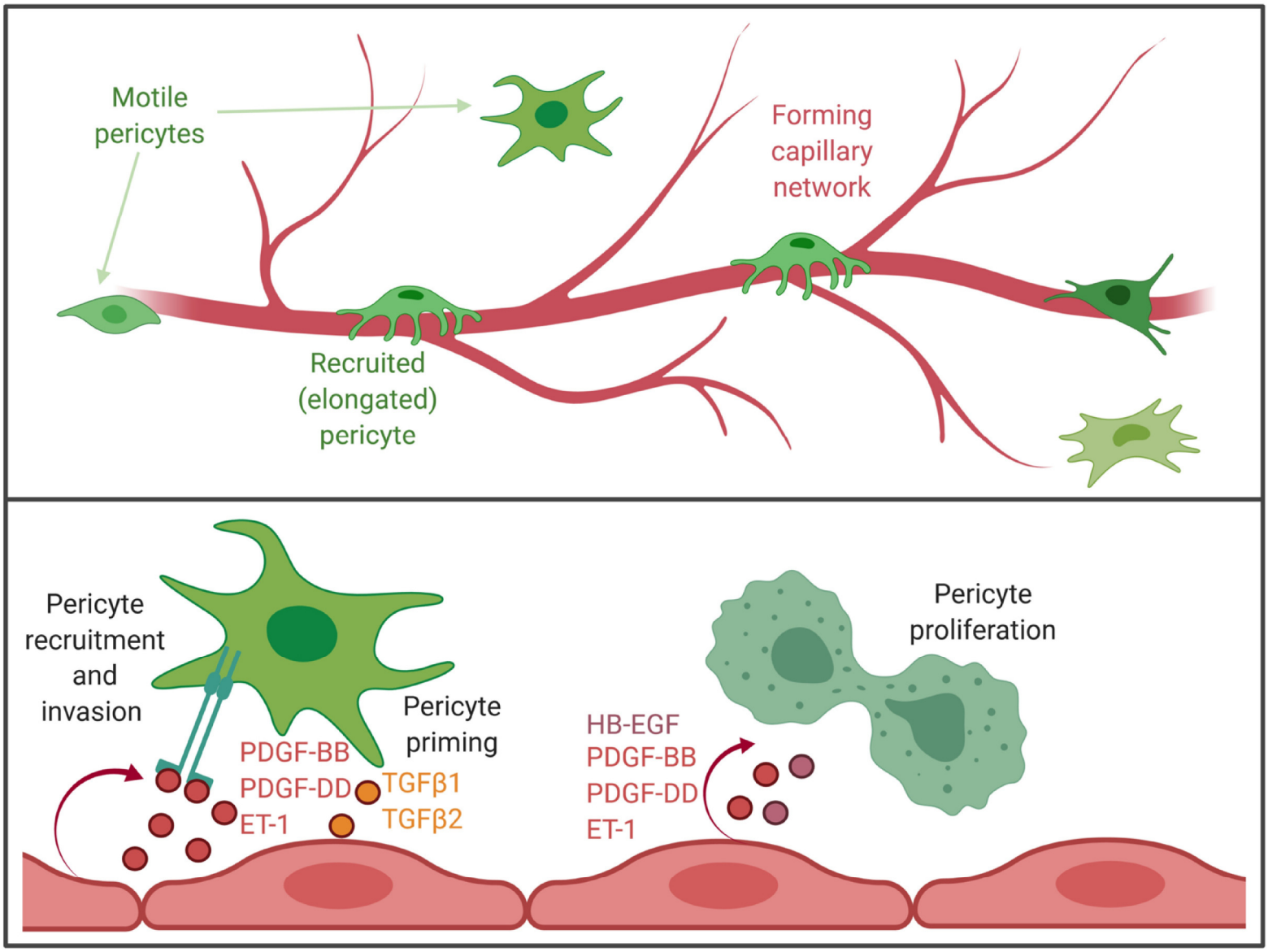
Figure legend. Schematic illustration of the paracrine EC-pericyte signalling that regulates pericyte invasion, proliferation and recruitment to EC tubes during capillary network formation. Created with BioRender.com


Motile
pericytes
Forming
capillary
network
Recruited
(elongated)
pericyte
Pericyte
recruitment
and
invasion
Pericyte
priming
PDGF-BB
PDGF-DD
ET-1
TGFβ1
TGFβ2
Pericyte
proliferation
HB-EGF
PDGF-BB
PDGF-DD
ET-1