Endothelial Cell Protein C Receptor
Role Beyond Endothelium?

Meenakshisundaram Thiyagarajan, Tong Cheng, Berislav V. Zlokovic

Recent evidence indicates that EPCR is expressed on different cells beyond aortic endothelial cells. For example, EPCR is expressed on the surface of monocytes, CD56+ natural killer cells, neutrophils and eosinophils, immature hematopoietic stem cells, brain capillary endothelial cells, EPCR is also expressed by embryonic giant trophoblast cells, and EPCR is critical for embryo development, because EPCR null mice die in mid-gestation. The study by Bretscher et al, in the present issue of Circulation Research demonstrates functionally active EPCR in systemic vascular smooth muscle cells (SMCs). The data show that EPCR mediates APC signaling in SMCs, resulting in increases in intracellular \([\text{Ca}^{2+}]\), phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK-1/2) and DNA synthesis (Figure 1). Along with EPCR, PAR-1 is also necessary to transduce APC signaling in SMCs.

These findings are consistent with previous work demonstrating that both EPCR and PAR-1 are required for APC-mediated activation of ERK1/2 and \([\text{Ca}^{2+}]\), signaling in systemic and cerebral capillary endothelial cells. These findings open a new chapter in biology of EPCR by demonstrating that EPCR can be expressed in cells which have different biological functions from endothelial and hematopoietic cells. In this context, it is of note that the microarray gene expression profiling of human cerebral SMCs and astrocytes have indicated the presence of EPCR mRNA and showed that the levels of EPCR transcripts in these brain cells were not altered by normal aging in humans or aging Alzheimer’s type (R. Zidovetski, N. Chow, B. Zlokovic, unpublished data, 2007). The exact role of EPCR and how its expression is regulated in nonendothelial cell types including systemic SMCs, primitive hematopoietic stem cells or cerebral SMCs, remain to be elucidated.

A regulatory element 5.5 kb upstream to the human EPCR gene (hEPCR) translation start site is essential for driving hEPCR expression in endothelium and primitive hematopoietic cells which share a common precursor. It would be interesting to find out whether the same site in hEPCR is responsible for driving hEPCR expression in human SMCs. The present findings by Bretscher et al raise not only a possibility that EPCR could play an important role in regulating the biology of vascular SMCs, but also suggest that its expression in SMCs might have implications for the pathogenesis of cardiovascular diseases such as atherosclerosis, and by extension, cerebral amyloid angiopathy in Alzheimer’s disease. EPCR may be a potential therapeutic target: novel EPCR agonists might modify cytoprotective APC/PC cellular pathways in injured or inflamed blood vessels.

We still have much to learn about the role of EPCR beyond the endothelium, and perhaps in the endothelium itself. For
example, Dr Esmon has suggested that EPCR appears to be able to translocate from the plasma membrane into the nucleus, a cellular trafficking pattern that is not observed with its Cys to Ser mutant.\textsuperscript{24} One may speculate that EPCR may internalize its endogenous ligands PC and APC into the cytoplasm and into the nucleus, which in turn could have important implications for regulation of gene expression and control of APC activity. Figure 2a shows dense punctate staining of APC on the surface of brain endothelial normoxic cells, which is completely removed by pretreatment of cells with an antibody that blocks APC binding site on EPCR (Figure 2b),\textsuperscript{25} demonstrating that APC binding to the cell is highly EPCR-dependent, as suggested.\textsuperscript{1} On the other hand, Figure 2c shows that APC can be transported intracellularly and into the nucleus of brain endothelial cells under hypoxic conditions (Figure 2c), which is associated with cell protection and survival, as reported.\textsuperscript{13} This transport pattern, however, completely disappears if APC binding to EPCR is blocked (Figure 2d), and cells die as a result of APC unavailability, as shown by faint APC staining on cell debris. Thus, the role of EPCR-APC-PAR-1-dependent intracellular cytoprotective signaling and of EPCR-mediated intracellular trafficking and internalization of APC/PC in normal and injured vascular endothelial cells and SMCs, may open a new era in our understanding of EPCR biology, its physiological and pathological functions, and therapeutic opportunities for
disorders of the cardiovascular system and cerebral circulation.

References


KEY WORDS: endothelial protein C receptor | activated protein C | protease activated receptor-1 | endothelium | vascular smooth muscle cells
Endothelial Cell Protein C Receptor: Role Beyond Endothelium?
Meenakshisundaram Thiyagarajan, Tong Cheng and Berislav V. Zlokovic

Circ Res. 2007;100:155-157
doi: 10.1161/01.RES.0000258167.48227.84

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/100/2/155