Ref-1 and Transcriptional Control of Endothelial Apoptosis

Nanping Wang, Michael B. Stemerman

Vascular endothelium, when unperturbed, provides a surface to the blood vessel, which is passive to the development of thrombosis, and potentially adherent blood cells. This characteristic is the quintessence of vascular homeostasis. However, endothelial cells (ECs) can undergo apoptosis in vitro in response to a variety of pathophysiologic conditions including hypoxia, proinflammatory cytokines, bacterial endotoxins, and atherogenic risk factors such as homocysteine and lipoproteins (reviewed in Stefanec and Dimmeler and Zeiher). These cellular perturbations have in common the generation of intracellular reactive oxygen intermediates, referred to as oxidative stress. ECs respond to these adverse conditions by altering their intracellular reduction/oxidation (redox) state and making their ultimate decision between adaptation (survival) and apoptosis (see Figure). Understanding the precise mechanisms controlling such a process is an important component to our knowledge of cardiovascular diseases. In this issue of Circulation Research, Hall et al provide novel evidence for a critical role of Ref-1, a redox-sensitive regulator, in affecting EC apoptosis.

Ref-1 was cloned as Redox factor, also known as apurinic (apyrimidinic) endonuclease (APE). As a ubiquitously expressed multifunctional 36-kDa protein, Ref-1 is involved in the repair of DNA damage as well as in the transcriptional regulation of genes. Its 5′AP-endonuclease functions in base excision repair, and its 3′-diesterase activity removes phosphoglycolate residues from DNA damaged by genotoxic stresses. In addition, Ref-1 is also important for the activation of transcription factors, such as activator protein-1 (AP-1), nuclear factor-κB (NF-κB), p53, and hypoxia-inducible factor-1α (HIF-1α). Activation of transcription factors, which occurs via a redox-based mechanism, pertains to its 6-kDa N-terminal domain. Following its discovery, Xanthoudakis and Curran identified Ref-1 as a reductive activator of c-Fos and c-Jun (two major components of AP-1) via a reduction of the conserved cysteine residues in their DNA binding domains. Interestingly, Ref-1 also acts as a transcriptional repressor of its own gene and other genes such as that coding for the parathyroid hormone. Although it has been observed that a decrease in Ref-1 protein level precedes apoptotic changes in rodent models for ischemic or traumatic brain injury, a role of Ref-1 in EC apoptosis has not been investigated previously. In the present study, hypoxia resulted in decrease in Ref-1 protein expression in both human umbilical vein ECs and bovine pulmonary artery ECs. Moreover, overexpression of Ref-1 rescued both hypoxia-and tumor necrosis factor (TNF-α)–induced apoptosis. This demonstrates that the decline in Ref-1 is a cause of, but not a response to, hypoxia-induced apoptosis. Further, Ref-1 appears to be an antiapoptotic factor in ECs. This agrees with a recent report showing a protective effect for Ref-1 in dopamine-induced neuron apoptosis.

Numerous agents are categorized as having pro- or anti-EC apoptotic properties. What remains as inconclusive and perhaps controversial are the roles of specific transcription factors controlling EC response to these various perturbations. Transcription factor NF-κB has, for years, been recognized as a central mediator of gene expression induced by proinflammatory cytokines and pathogens. It is thought to play a pivotal role in cardiovascular diseases including atherosclerosis (see review by Collins and Cybulsky). Activation of NF-κB has been linked to apoptosis, with the factor playing either an antiapoptotic or proapoptotic role, depending on the cell type. Activation of NF-κB is essential to protect TNF-α–induced apoptosis, which appears to be a common mechanism in many cell types. Although how NF-κB protects against apoptosis is far from established, it is believed that a major mechanism by which the transcription factor inhibits cell death is to induce the expression of antiapoptotic genes whose products, in turn, provide protection to the cells under adverse conditions. A number of such protective genes that are induced by NF-κB have been identified, including inhibitors of apoptosis (IAPs), TNF-receptor–associated factor-1 and –2 (TRAF-1 and TRAF-2), Bcl-2–like factors and A20, a zinc-finger protein that was originally identified as a TNF-inducible gene in ECs. Although NF-κB protects ECs from TNF-α–induced apoptosis, this survival pathway seems to provide little protection against some other proapoptotic stimuli such as lipopolysaccharide (LPS), interleukin-1β, and hypoxia, despite the fact that NF-κB is also activated in these scenarios. In addition, certain endothelial survival factors such as Bcl-2, Bcl-XL, and A20, which although suppressing NF-κB, can override cellular apoptotic signaling and make NF-κB dispensable in EC protection. Because NF-κB is a key transcription factor governing a variety of proinflammatory genes including chemokines and adhesion molecules, an NF-κB–dependent antiapoptotic pathway can protect endothelial integrity without converting the endothelium to a proinflammatory state. Such a mechanism is desirable for therapeutic intervention for many clinical conditions such as reperfusion injury and xenotransplantation. In the present study, Ref-1 rescues ECs from apoptosis via both an NF-κB–dependent and
Role of Ref-1 in endothelial apoptosis. Various pathophysiological conditions cause oxidative stress and intracellular redox change in ECs. Diverse signaling pathways activate transcription factors in both a cell type- and context-specific manner. Successive regulation of pro- or antiapoptotic gene expression controls EC apoptosis or survival. As a DNA repair protein (exhibiting 5'AP-endonuclease activity and 3'-phosphodiesterase) and a regulator of transcription (via redox-based activation of transcription factors [e.g., AP-1, p53, and NF-κB]), Ref-1 may play a pivotal role in modulating EC fate under oxidative stress.

What appears to be more paradoxical is that Ref-1 is also known as a potent activator for the tumor suppressor p53, which, when activated in cells, can induce either cell cycle arrest or apoptosis. The p53 is activated in response to genotoxic stresses and is associated with hypoxia-induced EC death. Gaidd on et al recently showed that Ref-1 enhances the proapoptotic functions of p53 in a transformed cell line. It thus seems to be an apparent contradiction that in the present study ECs were prevented from undergoing apoptosis by overexpression of Ref-1, which, on the other hand, may activate p53. However, it should be pointed out that, in the study of Gaiddon et al, Ref-1 increased the ability of p53 to induce apoptosis only when exogenous p53 and Ref-1 were both overexpressed by cotransfection. Thus, it is unclear whether Ref-1 activation of endogenous p53 is to induce apoptosis or, alternatively, to arrest cell cycle. In fact, laminar shear stress, known to promote EC survival, can cause sustained activation of p53 and endothelial growth arrest. Given that Ref-1 possesses dual functions as transcriptional regulator and DNA repair enzyme, it is rational to speculate that these two domains of Ref-1, although they can function independently, may act in concert to protect cells from oxidative damage: one activates p53 to ensure efficient cell-cycle arrest for the other to fix the DNA damage. Nevertheless, precise interactions between Ref-1 and certain transcription factors as well as their functional readouts under specific endothelial conditions would be of considerable importance in understanding the transcriptional regulation of EC apoptosis. The need to understand this effect of Ref-1 is underscored by the increasing number of transcription factors that have been found to interact with Ref-1. On this expanding list are HIF-1α, HIF-like factor (HLF), activating transcription factor (ATF), cAMP response element–binding protein (CREB), the oncogene Myb, nuclear factor-Y (NF-Y), and early growth response-1 gene (Egr-1), Pax-5, and Pax-8. Although the consequences of activating these transcription factors remain poorly understood, it can be hypothesized that Ref-1 may play a pivotal role in integrating the transcriptional response and, thus, control EC fate under specific oxidative conditions. However, an important caveat must be considered regarding the pathogenetic importance of EC apoptosis. Most reports studying EC programmed cell death have examined the process in cell culture. Although a few studies have shown in situ detection of EC apoptosis in microvessels and transplant coronary artery disease, it is yet uncertain as to its importance in major circulatory disorders such as atherosclerosis. Until such in vivo studies are carried out, the role for EC apoptosis in vascular diseases remains speculative.

Finally, the finding that Ref-1 increases EC survival under conditions of hypoxia and TNF-α stimulation has potential clinical relevance to vascular diseases. For example, upregulation of Ref-1 by either gene transfer or pharmacological agonists can be expected to promote angiogenesis that is therapeutically desirable for ischemic diseases and wound healing. On the other hand, antagonizing Ref-1 may exert an angiostatic effect and, in turn, inhibit tumor growth.

References
5. Dempfle B, Herman T, Chen DS. Cloning and expression of APE, the cDNA encoding the major human apurinic endonuclease: definition of a

KEY WORDS: endothelium □ apoptosis □ transcription factor □ oxidative stress
Ref-1 and Transcriptional Control of Endothelial Apoptosis
Nanping Wang and Michael B. Stemerman

Circ Res. 2001;88:1223-1225
doi: 10.1161/hh1201.093162

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 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/88/12/1223

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/