ABIN-2 protects endothelial cells from death and has a role in the antiapoptotic effect of angiopoietin-1

Amir Tadros, David P. Hughes, Benjamin J. Dunmore, and Nicholas P. J. Brindle

Introduction

The receptor tyrosine kinase Tie2 is expressed primarily in endothelial cells and is essential for blood vessel formation. A family of ligands, the angiopoietins, has been identified for Tie2. Angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) are the best-characterized members of this ligand family. Ang1 activates Tie2 whereas Ang2 can antagonize this effect. Activation of Tie2 stimulates endothelial cell migration and sprouting, and the receptor is required for correct organization and integrity of the vascular system during development. In addition, Ang1 stimulation of Tie2 inhibits vascular leakage in the adult and suppresses vascular inflammation. These effects point to an important vascular protective activity of the Ang1/Tie2 system. In accord with this, angiopoietin stimulation of Tie2 also inhibits endothelial cell apoptosis in response to growth factor deprivation, irradiation, and mannitol treatment.

Recently Tie2 was found to interact with the protein A20 binding inhibitor of NF-κB activation (ABIN-2) in a ligand-dependent manner. As the name suggests, ABIN-2 is an inhibitor of nuclear factor-κB (NF-κB) and it appears to have a role in Ang1 suppression of NF-κB activity. As well as regulating inflammatory gene expression, NF-κB has antiapoptotic activity in many cell types. In endothelial cells, NF-κB activity is necessary for prevention of apoptosis induced by tumor necrosis factor-α (TNF-α) and growth factor deprivation. Although ABIN-2 inhibits NF-κB, its effects on apoptosis are not known and its involvement in Tie2-mediated endothelial survival is unexplored.

ABIN-2 and the related protein ABIN-1 were originally discovered as binding partners for the inducible protein A20. This zinc finger protein is a potent inhibitor of NF-κB activity with a key role in limiting the extent and duration of inflammatory activation. ABIN-2 has been postulated as one of the mediators of the inhibitory effect of A20 on NF-κB. A20 inhibits apoptosis in endothelial cells and some other cell types. Expression of A20 inhibits apoptosis induced by growth factor deprivation of human umbilical vein endothelial cells (HUVECs) and by TNF-α in HUVECs and lipopolysaccharide in human microvascular endothelial cells. It is possible, therefore, that ABIN-2, like A20, could both inhibit NF-κB and suppress cell death. Such activity would make ABIN-2 an attractive target for promoting cytoprotection of the endothelium. In the present study we examine the effects of ABIN-2 on apoptosis of endothelial cells and its potential involvement in Tie2-mediated endothelial survival.

Study design

The expression vector encoding green fluorescent protein (GFP) was obtained from BD Biosciences Clontech (Palo Alto, CA). Isolation and culture of HUVECs, preparation of the recombinant version of Ang1, Ang1*, and the generation of cDNA encoding human ABIN-2 were as described previously. Endothelial cells were transfected using Targeffect F2 transfection reagent (Targeting Systems, Santee, CA). For examination of endothelial apoptosis and survival, HUVECs were grown to 80% to 90% confluence on gridded tissue culture dishes and transfected with control or test plasmids together with GFP. Twenty-four hours after transfection, cells were washed and incubated in growth factor– and serum-free medium for 18 hours before analysis of the transfected cells—assessed by expression of GFP—for apoptotic index or cell survival. Apoptotic index was determined 18 hours before analysis of the transfected cells—assessed by expression of GFP—for apoptotic index or cell survival. Apoptotic index was determined 18 hours before analysis, media were removed and cells fixed for 10 minutes at room temperature in 4% formaldehyde in phosphate-buffered saline (PBS). Nuclei were stained with 4,6 diamidino-2-phenylindole (DAPI) at 0.1

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ABIN-2 exhibited a significant increase in percentage survival of growth factor-deprived endothelial cells (Figure 1C). The protein kinases phosphatidylinositol-3 kinase (PI-3K) and Akt have central roles in protecting endothelial and other cells from death. Expression of ABIN-2 increased the activated phospho-Ser473 form of Akt (Figure 1E), and the PI-3K inhibitors wortmannin and LY294002 inhibited ABIN-2–induced endothelial survival (Figure 1F), suggesting a role for the PI-3K/Akt pathway in the prosurvival activity of ABIN-2.

Results and discussion

Although A20 has been shown to inhibit cell death in a number of cell types, including growth factor–deprived endothelial cells, the effects of the A20 binding protein and putative NF-kB effector ABIN-2 on apoptosis have not been explored. To examine the potential antiapoptotic activity of ABIN-2, HUVECs were transfected with expression plasmids encoding GFP and control plasmid or with GFP and ABIN-2. Twenty-four hours after transfection, endothelial cells were growth factor–deprived for 18 hours before assessment of apoptotic index in transfected cells (Figure 1A). As previously documented, growth factor deprivation of HUVECs induces substantial apoptotic cell death. However, cells expressing ABIN-2 exhibited a significant 2-fold decrease in apoptotic index (Figure 1B). Consistent with this, the cleaved 17 and 19 kDa active forms of caspase-3 were present in growth factor–deprived HUVECs and decreased by expression of ABIN-2 (Figure 1C). As might be expected from its effects on apoptotic index, expression of ABIN-2 also caused a significant increase in percentage survival of growth factor–deprived endothelial cells (Figure 1D). The protein kinases phosphatidylinositol-3 kinase (PI-3K) and Akt have central roles in inducing substantial apoptotic cell death. However, cells expressing ABIN-2 also caused a significant increase in percentage survival of growth factor deprivation.
Deletion of the carboxy-terminus of ABIN-2 prevents it from inhibiting stimulated NF-κB activity when expressed in human embryonic kidney cells. We therefore examined whether deletion of the carboxy-terminal 85 amino acid residues affects the ability of ABIN-2 to prevent endothelial cell death. This deleted form of ABIN-2 was expressed in endothelial cells and its ability to promote survival examined as before. The ability of expressed ABIN-2 to rescue endothelial cells from death was lost on removal of the carboxy-terminal sequence of the protein (Figure 2A).

ABIN-2 has recently been shown to interact with the endothelial receptor tyrosine kinase Tie2. Activated Tie2 inhibits endothelial cell death. We were interested, therefore, to determine whether ABIN-2 has a role in the antiapoptotic activity of Tie2. To examine this we utilized the carboxy-terminal–deleted form of ABIN-2 as a putative dominant negative. The ability of the Tie2 agonist Ang1 to promote survival of growth factor–deprived endothelial cells was examined in cells expressing control vector or deleted ABIN-2. Ang1 caused a significant increase in survival of growth factor–deprived endothelial cells expressing control vector (Figure 2B). In contrast, expression of the truncated form of ABIN-2 interfered with the ability of Ang1 to promote endothelial survival consistent with ABIN-2 involvement in the prosurvival activity of Tie2.

The PI-3K/Akt pathway has been shown to have an essential role in Tie2-mediated endothelial survival. The current findings support a role also for ABIN-2 in the prosurvival activity of Tie2. The exact relationship between the PI-3K/Akt pathway and ABIN-2 in inhibition of endothelial apoptosis will require definition of the mechanism by which ABIN-2 promotes survival of endothelial cells.

In summary, these data demonstrate for the first time that ABIN-2 has antiapoptotic activity in endothelial cells and that this protein is involved in Ang1 inhibition of endothelial apoptosis. This prosurvival activity, together with its known antiinflammatory effects, suggests ABIN-2 may be an important vascular protective protein.

References

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