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Regulation of apoptosis by protein S-nitrosylation

Review Article

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Summary. S-nitrosylation/denitrosylation of critical cysteine residues on proteins serves as a redox switch that regulates the function of a wide array of proteins. A key signaling pathway that is regulated by S-nitrosylation is apoptotic cell death. Here we will review the proteins in apoptotic pathways that are known to be S-nitrosylated by endogenous NO production. The targets and functional consequences of S-nitrosylation during apoptosis are multifaceted, allowing cells to fine tune their response to apoptotic signals.

Keywords: S-nitrosylation – Apoptosis – Nitric oxide – Caspases – GAPDH – NMDA – Thioredoxin, Ask1, FLIP

Introduction

S-Nitrosylation is the modification of a cysteine thiol on a protein or peptide by a nitric oxide (NO) group. The mechanisms underlying S-nitrosothiol (SNO) formation in vivo are not understood and may not involve direct reactions between NO and thiols. Instead, formation of SNOs from NO may require the presence of electron acceptors such as oxygen, type 1 copper or iron to generate NO^+ equivalents that react with thiolate anions, or thiyl radicals that react with NO (Gaston et al., 2003). Like phosphorylation, S-nitrosylation is a specifically targeted and rapidly reversible posttranslational modification that regulates protein function during cell signaling (Lane et al., 2001; Mannick and Schonhoff, 2002). Although many proteins have been shown to be S-nitrosylated in cell free systems or when cells are exposed to exogenous NO donor compounds, it has been much more difficult to identify proteins that are S-nitrosylated intracellularly by endogenous sources of NO. Identification of endogenously S-nitrosylated proteins is technically challenging because their intracellular concentration is often at the limits of detection of currently available technology. In addition, the S–NO bond is redox sensitive and subject to allosteric control. Therefore, S–NO bonds can be lost during sample preparation as a result of exposure to factors such as UV light, reducing agents, pH and pO_2 shifts, and low level transition metal contamination of buffers (Hess et al., 2005). Despite these methodological constraints, an increasing number of proteins have been shown to be regulated by endogenous intracellular S-nitrosylation including G proteins, kinases, transcription factors, receptors and proteases (Hess et al., 2005). A key signaling pathway that is regulated by endogenous S-nitrosylation is apoptotic cell death. Here we will review proteins that are regulated by endogenous S-nitrosylation during apoptosis. In general, S-nitrosylation resulting from basal low level NO production in cells has anti-apoptotic effects. S-nitrosylation resulting from higher level stimulated NO production has either pro-apoptotic effects or serves as a negative feedback mechanism to downregulate apoptotic responses.

Inhibition of apoptosis by caspase S-nitrosylation

Caspases are a family of cysteine proteases that play an essential role in the initiation and execution of apoptotic pathways. Caspases are expressed as inactive zymogens in resting cells. During apoptosis, caspase zymogens are autocleaved or cleaved by other caspases to form active tetrameric enzymes. All caspases have a critical catalytic site cysteine that is a target of S-nitrosylation. Basal S-nitrosylation of the catalytic site cysteine inhibits cas-

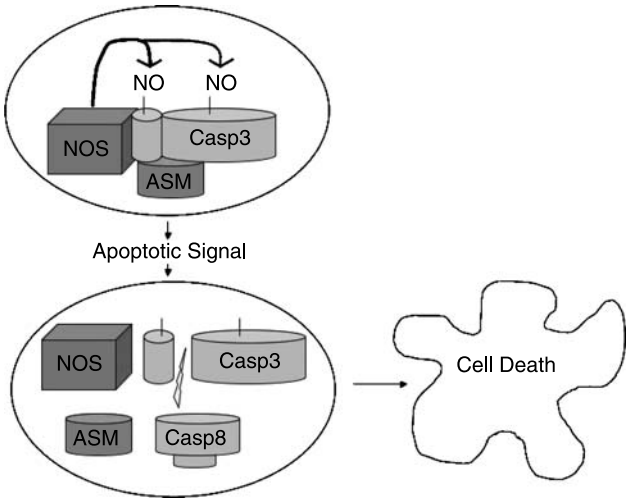

Fig. 1. Inhibition of apoptosis by caspase-3 S-nitrosylation. In resting cells caspase-3 zymogens are targeted for S-nitrosylated via an association with NOS. S-nitrosylation of the catalytic site cysteine inhibits the activity of caspase-3 in resting cells. S-nitrosylation of an additional cysteine residue promotes the association of caspase-3 with ASM and thereby inhibits the ability of upstream caspases such as caspase-8 to cleave and activate caspase-3. After cells receive an apoptotic signal, caspase-3 zymogens are denitrosylated. Denitrosylation activates the catalytic site cysteine and causes ASM to dissociate from caspase-3. Caspase-3 is then cleaved by capase-8 and apoptotic cell death ensues

pase activity and apoptosis (Dimmeler et al., 1997; Kim et al., 1997; Li et al., 1997; Mannick et al., 1999). We have found that in human lymphocyte cell lines, it is predominantly the subpopulation of caspases residing in mitochondria that is S-nitrosylated (Mannick et al., 2001). S-nitrosylation of a second cysteine may also lead to an association of a caspase-3 with acid sphingomyelinase (ASM). The association of ASM with S-nitrosylated caspase-3 further inhibits apoptosis by inhibiting the cleavage and activation of caspase-3 by upstream caspases (Matsumoto et al., 2003). After a variety of apoptotic stimuli, caspases are denitrosylated (Kim and Tannenbaum, 2004; Mannick et al., 1999), allowing the catalytic site to function and triggering the dissociation of ASM with caspase-3. As a result, caspase activity increases and triggers apoptotic cell death (Fig. 1). Thus, S-nitrosylation of caspases by basal intracellular NO production inhibits caspase activation and apoptosis in resting cells, whereas denitrosylation activates caspases and promotes apoptosis.

Inhibition of apoptosis by thioredoxin S-nitrosylation

The oxidoreductase thioredoxin 1 (Trx) is also S-nitrosylated by basal intracellular NO production. Endogenous S-nitrosylation of Trx on cysteine 69 inhibits TNF-α-induced apoptosis (Haendeler et al., 2002). The mechanism by which S-nitrosylation enhances the anti-apoptotic activity of Trx is not known. However, S-nitrosylation increases the oxidoreductase activity of Trx which may inhibit apoptosis by limiting free radical production in cells. It is also possible that S-nitrosylated Trx inhibits apoptosis by transferring its NO group to cysteines on proteins such as caspases (Haendeler et al., 2002).

Co-localization of NOS with S-nitrosylation targets

Since high concentrations of NO induce apoptosis, anti-apoptotic effects of NO are generally seen when NO is produced at very low basal levels in cells. The question then arises, how do such low levels of intracellular NO production result in S-nitrosylation of specific proteins involved in apoptotic cell death? Recent data suggests that some proteins are targeted for S-nitrosylation by co-localization with nitric oxide synthase (NOS) in cells. Co-localization may result from direct associations of proteins with NOS (Matsumoto et al., 2003), from associations with adapter proteins in NOS-containing multi-protein complexes (Fang et al., 2000), or from compartmentalization of proteins with NOS in subcellular microdomains such as the mitochondrial intermembrane space (Mannick et al., 2001). For instance, caspase-3 may be specifically targeted for S-nitrosylation because it directly associates with all 3 NOS isoforms (Matsumoto et al., 2003). Thus, although the total intracellular level of NO production may be very low in resting cells, the local concentration of NO produced by NOS may be much higher and facilitate S-nitrosylation of cysteine residues on adjacent proteins. Whether multiple proteins in apoptotic pathways are targeted for S-nitrosylation due to co-associations with NOS remains to be determined.

S-nitrosylation as a negative feedback mechanism in apoptosis

Although NO production tends to be very low in resting cells, apoptotic stimuli often increase NOS activity and NO production (Hara et al., 2005; Chanvorachote et al., 2005). In some instances, NOS stimulation results in protein S-nitrosylation that serves as a negative feed back loop to inhibit apoptosis. For instance, interferon-γ-induced increases in NO production down regulate apoptosis via S-nitrosylation of apoptosis signal-regulating kinase 1 (ASK1), a protein that stimulates apoptosis when activated (Park et al., 2004). S-nitrosylation of ASK1 on cysteine 869 inhibits ASK1 activation by decreasing the binding of ASK1 to its substrate kinases MKK3 or MKK6

(Park et al., 2004). Similarly, stimulation of the NMDA (*N*-methyl-D-aspartate) class of glutamate receptor on neurons leads to increased NOS activation (Garthwaite et al., 1988) that down regulates NMDA receptor activity and apoptosis via S-nitrosylation of the NR2a regulatory subunit of the NMDA receptor (Choi et al., 2000; Lipton et al., 1993). Likewise, Fas stimulation results in increased NO production that down regulates apoptosis via S-nitrosylation of cysteines 254 and 259 on FLICE inhibitory protein (FLIP) (Chanvorachote et al., 2005). FLIP inhibits apoptotic signaling by interfering with the binding of caspase-8 to the adaptor protein FADD at the Fas receptor complex. S-nitrosylation of FLIP after Fas stimulation inhibits the ubiquitination and proteasomal degradation of FLIP. As a result, FLIP levels are increased and caspase-8 activation and apoptosis are inhibited (Chanvorachote et al., 2005).

Subcellular compartmentalization of S-nitrosylation/denitrosylation

Of interest, Fas stimulation results both in the denitrosylation of caspase-3 zymogens in mitochondria and the S-nitrosylation of FLIP in the cytosol or cytoplasmic membrane. These findings underscore the importance of subcellular compartmentalization in determining if, when and where proteins are S-nitrosylated during signal transduction. For instance, it is possible that NO production in mitochondria decreases after Fas stimulation whereas NO production in the cytosol increases. It is also possible that NO production increases in both subcellular compartments but that denitrosylase activity increases in mitochondria. Thus levels of protein S-nitrosylation will depend not only on the rate of NO production and S-nitrosothiol synthesis in a given subcellular compartment, but also on the local denitrosylase activity.

Pro-apoptotic effects of GAPDH S-nitrosylation

Higher levels of NO production induced by apoptotic stimuli also result in protein S-nitrosylation that is pro-apoptotic. For instance, a variety of apoptotic stimuli lead to increased NO production and subsequent S-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Hara et al., 2005). S-nitrosylation of GAPDH augments its binding to the E3 ubiquitin ligase Siah1, whose nuclear localization signal mediates the nuclear translocation of GAPDH. In the nucleus, GAPDH stabilizes Siah1, facilitating its degradation of nuclear proteins and stimulating apoptotic cell death (Hara et al., 2005) (Fig. 2).

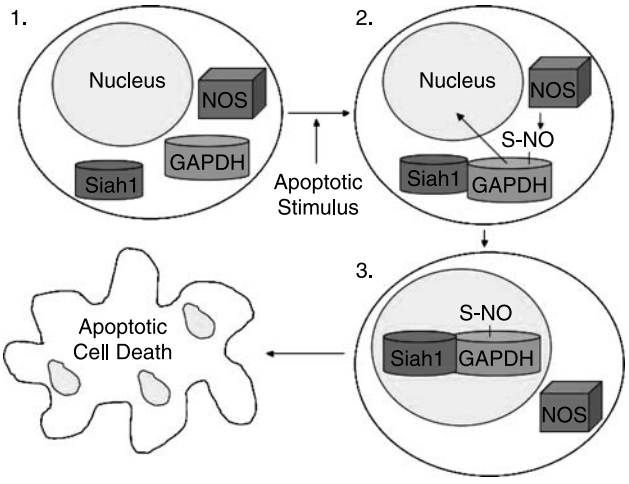

Fig. 2. S-nitrosylation of GAPDH stimulates apoptosis. In resting cells, GAPDH is found predominantly in the cytoplasm. In response to an apoptotic stimulus, GAPDH is S-nitrosylated as a result of NOS activation. S-nitrosylated GAPDH associates with Siah1, whose nuclear localization signal mediates the nuclear translocation of GAPDH. In the nucleus, GAPDH stabilizes Siah1, facilitating its degradation of nuclear proteins and stimulating apoptotic cell death

Contributions of S-nitrosylation to cell death in disease

In some instances increased NO production and protein S-nitrosylation contribute to cell death in disease. For instance, during cerebral ischemia, matrix metalloproteinase-9 (MMP-9) colocalizes with nNOS and is S-nitrosylated on a critical cysteine residue. The cysteine normally coordinates a zinc ion that blocks the catalytic site of MMP-9 (Gu et al., 2002). However, S-nitrosylation leads to the formation of a stable sulfinic or sulfonic acid derivative of the cysteine that disrupts the cysteine-zinc coordination and causes irreversible activation of MMP-9. As a result, extracellular proteolytic cascades are triggered that disrupt the extracellular matrix and contribute to stroke formation (Gu et al., 2002).

Similarly, increased NO production during Parkinson's disease leads to S-nitrosylation of parkin (Chung et al., 2004). Parkin is an E3 ubiquitin ligase involved in the ubiquitination of proteins. Inhibition of parkin activity by S-nitrosylation may contribute to the apoptotic death of dopamine neurons in Parkinson's disease by allowing abnormally folded or damaged proteins to accumulate intracellularly (Chung et al., 2004).

Conclusion

In conclusion, S-nitrosylation has both positive and negative regulatory roles during apoptosis. In general, low-

level basal S-nitrosylation of proteins exerts anti-apoptotic effects. In contrast, higher level NO production induced by apoptotic stimuli leads to protein S-nitrosylation that either promotes apoptosis or serves as a negative feedback loop and inhibits apoptosis. Regulation of apoptotic signaling by S-nitrosylation is complex, and in the same signaling pathway, some proteins may be S-nitrosylated and others denitrosylated depending on their subcellular localization. In addition, S-nitrosylation of some proteins may stimulate apoptosis whereas S-nitrosylation of other proteins may inhibit apoptosis. For instance after NMDA stimulation, increased S-nitrosylation of GAPDH stimulates apoptosis whereas increased S-nitrosylation of the NMDA receptor down regulates receptor activity and apoptosis (Choi et al., 2000; Hara et al., 2005; Lipton et al., 1993). The multiple stimulatory and inhibitory targets of S-nitrosylation may allow cells to fine tune their response to apoptotic signals. Finally, dysregulated protein S-nitrosylation may contribute to cell death in pathologic conditions such as stroke and Parkinson's disease (Chung et al., 2004; Gu et al., 2002; Yao et al., 2004). Elucidation of the specific targets and functional consequences of S-nitrosylation during apoptosis may lead to the development of novel NO-based therapies for diseases such as cancer and neurodegeneration that are associated with deficient or excessive apoptotic cell death.

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