ATM Gene Regulates Oxygen-Glucose Deprivation–Induced Nuclear Factor-κB DNA-Binding Activity and Downstream Apoptotic Cascade in Mouse Cerebrovascular Endothelial Cells

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Background and Purpose—Cells lacking the ATM (ataxia telangectasia mutated) gene are hypersensitive to DNA damage caused by a variety of insults. ATM may regulate oxidative stress–induced signaling cascades involving nuclear factor-κB (NF-κB), a transcription factor that is upstream of a wide variety of stress-responsive genes. We investigated the potential interaction of ATM and NF-κB after oxygen-glucose deprivation (OGD) in cerebral endothelial cells (CECs).

Methods—Primary cultures of mouse CECs were subjected to OGD in the absence or presence of ATM antisense oligonucleotides or the NF-κB inhibitor SN50. ATM expression was determined with the use of reverse transcription–polymerase chain reaction and Western blot, and NF-κB activity was assessed by electrophoretic mobility shift assay. Cells were assessed for mitochondrial DNA damage with the use of long polymerase chain reaction and were assessed for caspase-3 and caspase-8 activity with the use of fluorogenic substrates. Cell death was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide and LDH release.

Results—OGD stimulated ATM gene expression at the mRNA and protein level in CECs as early as 1 hour after OGD initiation. ATM gene knockdown with the use of an antisense oligonucleotide suppressed OGD-induced ATM protein expression, which was accompanied by an attenuation of NF-κB activation and the subsequent expression of downstream genes, including the antiapoptotic gene c-IAP2. ATM knockdown also accentuated OGD-induced mitochondrial DNA damage and the activation of caspase-3 and caspase-8, leading to enhanced CEC death. The specific NF-κB inhibitor SN50 mimicked the effects of ATM knockdown.

Conclusions—We conclude that ATM may play a cytoprotective role in OGD-induced CEC death via a NF-κB–dependent signaling pathway. (Stroke. 2002;33:2471-2477.)

Key Words: ataxia telangectasia • cell death • cerebral cortex • endothelium, vascular • genetics • glucose • NF-kappa B • oxygen

An important early response to cellular stress involves the activation of nuclear transcription factors. Prominent among these factors is nuclear factor-κB (NF-κB), a central regulator of a diverse array of stress-responsive genes, which is activated in response to a wide variety of stimuli, including oxidative stress, infections, cytokines, and hypoxia/ischemia. Reactive oxygen species have been implicated in the activation of NF-κB, as shown in experimental paradigms that heightened oxidative stress. NF-κB exists as a heterodimer consisting of p65/RelA and p50/NFκB1 and is inactive in the cytoplasm when bound to IκB proteins, which obscure a nuclear localization signal. NF-κB activation requires IκB phosphorylation, marking it for degradation, thereby relieving its inhibitory hold. Activated NF-κB is then free to translocate to the nucleus, where it can bind to its cognate DNA binding sites to transactivate downstream genes. While genes regulated by NF-κB activation are diverse and complex, accumulating evidence suggests that one of its roles may involve cytoprotective action against apoptosis induced by oxidative stress and other death signals. NF-κB activation prevented apoptosis in several death paradigms. One proposed mechanism for this antiapoptotic action is NF-κB transactivation of a downstream gene that encodes c-IAP2 (inhibitor of apoptosis protein).

ATM (ataxia telangectasia mutated) may be involved in regulating stress-induced NF-κB expression. The ATM gene codes for a 350-kDa protein containing a phosphatidylinositol 3-kinase enzymatic and DNA-binding domain.
which binds directly to free DNA ends and activates kinases involved in selected signaling cascades. These properties of ATM suggest a role as a DNA damage sensor to transmit signals to checkpoints and DNA repair proteins.\textsuperscript{11} Mutational inactivation of the ATM gene leads to the congenital disease ataxia telangiectasia (AT).\textsuperscript{9,12} AT is characterized by multi-organ abnormalities, including dysfunction in the nervous, vascular, and immune systems.\textsuperscript{13,14} ATM-deficient cells are more susceptible than wild-type cells to oxidative damage\textsuperscript{15,16} and apoptosis.\textsuperscript{17} Inactivation of IxB-\(\alpha\) in ATM-deficient cells ameliorated this vulnerability, suggesting the involvement of NF-\(\kappa\)B in this disorder.\textsuperscript{18} Indeed, ATM is required for the degradation of IxB-\(\alpha\) to activate NF-\(\kappa\)B after exposure to oxidative stress.\textsuperscript{19} One of the characteristic clinical findings in AT patients is telangiectasias, which develops between 3 and 6 years of age most notably on the bulbar conjunctivae but which is also present throughout the dermis (especially sun-exposed areas).\textsuperscript{20} Vascular abnormalities have also been described in the meninges and brain\textsuperscript{21} of older AT patients. Thus, ATM may play a role in the survival and normal development of vascular cells. To explore the potential interaction of ATM and NF-\(\kappa\)B under conditions of oxidative stress, we studied knockdown of the ATM gene in cultured cerebral endothelial cells (CECs) subjected to oxygen-glucose deprivation (OGD). NF-\(\kappa\)B activation has been reported in human CECs subjected to hypoxia/ischemia.\textsuperscript{22,23} In this report we present evidence that ATM is upregulated in CECs after OGD and that it may exert a protective effect on these cells via NF-\(\kappa\)B activation and subsequent antiapoptotic activity.

**Materials and Methods**

All chemicals and reagents were purchased from Sigma-Aldrich, and all cell culture supplies were purchased from Invitrogen Corporation unless specified otherwise.

**Cell Culture**

Mouse CECs were prepared as previously described.\textsuperscript{24} Briefly, mouse cerebral cortex was homogenized, filtered, and sequentially digested with collagenase B, then collagenase/disparse (Roche Molecular Biochemicals), followed by centrifugation in a 40% Percoll solution. The second band containing microvessels was collected and digested with collagenase B, then collagenase/dispase (Roche). Mouse cerebral cortex was homogenized, filtered, and sequentially digested with collagenase B, then collagenase/dispase (Roche). Mouse cerebral cortex was homogenized, filtered, and sequentially digested with collagenase B, then collagenase/dispase (Roche). Mouse cerebral cortex was homogenized, filtered, and sequentially digested with collagenase B, then collagenase/dispase (Roche).

**Oxygen-Glucose Deprivation**

Confluent mouse CECs were transferred into a temperature-controlled (37°C) anaerobic chamber (Forma Scientific) containing a gas mixture composed of 5% CO\(_2\), 10% H\(_2\), 85% N\(_2\), and 0.02% to 0.2% O\(_2\).\textsuperscript{26} The culture medium was replaced with oxygenated glucose-free Hanks’ balanced salt solution, and cells were maintained in the hypoxia chamber for 4 hours. Control mouse CECs were not exposed to OGD.

**Treatment With ATM Antisense Oligonucleotide or SN50**

In some experiments, mouse CECs were treated with 30 \(\mu\)g/mL SN50 (Biomol Research Laboratory Inc) for 24 hours before OGD. A separate group of CECs was treated with antisense or sense Morpholino oligonucleotide to ATM according to the protocol provided by the manufacturer.\textsuperscript{27} The oligonucleotides were custom-made by Gene Tools LLC, with the following antisense sequence: 5’-GTGCTAGACTCATGGTTTAAGATT-3’. Mouse CECs were incubated in a 5-mL solution consisting of 1.4 \(\mu\)mol/L antisense or sense ATM oligonucleotides and 0.56 \(\mu\)mol/L ethoxylated polyethyleneimine for 3 hours and subsequently switched to normal growth medium for at least 24 hours before exposure to OGD for 4 hours. We chose our analysis at 4 hours after OGD onset, a time when approximately 30% of cells die and when ATM is induced.\textsuperscript{26}

**Western Blot Analysis**

Cytoplasmic and nuclear proteins were isolated from CECs as described previously,\textsuperscript{26,28} and subjected to 6% to 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a polyvinylidene difluoride membrane. The blot was incubated with the following primary antibodies for 1 to 2 hours: rabbit anti-ATM antibody (1:100; Oncogene), rabbit anti-cIAP2 antibody (1:250), goat anti-survivin antibody (1:250), mouse anti–intercellular adhesion molecule-1 (ICAM-1) antibody (1:100, Dako Corporation), and mouse anti-actin antisemur (1:500). The membrane was then incubated with the secondary antibody (1:5000; anti-rabbit, anti-mouse, or anti-goat IgG conjugated with alkaline phosphatase, Promega) at room temperature for 1 hour, and the reaction product was developed according to the Promega protocol.

**Reverse Transcription–Polymerase Chain Reaction**

ATM gene mRNA expression was detected by reverse transcription–polymerase chain reaction (RT-PCR). Total RNA was isolated with RNeasy Mini Kit (Qiagen). Equal amounts of total RNA (600 ng) were reverse-transcribed with 1 \(\mu\)mol/L oligo(dT) and 0.5 mmol/L dNTPs (Qiagen), 10 U RNasin (Promega), and 4 U reverse transcriptase (Qiagen) for 1 hour at 37°C. cDNA was amplified in 0.2 mmol/L dNTPs, 1 \(\mu\)mol/L of each primer, 1.5 mmol/L MgCl\(_2\), and 2.5 U Taq polymerase (Roche). PCR was performed for 26 cycles alternating between 95°C for 20 seconds and 53°C for 30 seconds, followed by extension at 72°C for 1 minute. Primers were designed on the basis of the mouse ATM sequences (forward: 5’-ACTGGTGTGAAGTA-TTCACGC-3’; reverse: 5’-TGACAGCCGACCTGTTGAG-3’). The amplified products were analyzed on 1% agarose gel. The relative mRNA level of ATM was normalized to endogenous cyclophilin mRNA for each sample. The RT-PCR was conducted within the linear ranges of PCR cycles and RNA input. The PCR experiments were repeated 3 times, each using separate sets of cultures.\textsuperscript{26}

**Electrophoretic Mobility Shift Assay**

Gel shift assay to assess NF-\(\kappa\)B binding activity has been described in detail elsewhere.\textsuperscript{26} The following NF-\(\kappa\)B consensus oligonucleotide was used: 5’-AGTGGGACCCACATTCTCCAGG-3’ (Promega). The NF-\(\kappa\)B oligonucleotide was labeled with \(\gamma\)-\(\text{P}\)–ATP, and the binding reaction was performed in a total volume of 20 \(\mu\)L containing 5X binding buffer, 0.0175 pmol of labeled probe (>10 000 cpm), 20 \(\mu\)g of nuclear protein, and 1 \(\mu\)g of poly dIdC. After incubation for 20 minutes at room temperature, the mixtures were subjected to electrophoresis on a nondenaturing 6% polyacrylamide gel at 180 V for 2 hours under low ionic strength conditions. The gel was dried and subjected to autoradiography.

**Long PCR for Quantitation of Mitochondrial DNA**

The quantification of intact mitochondrial DNA (mtDNA) was achieved with the use of a previously described long PCR method with modifications.\textsuperscript{28,30} The Long PCR method yields reliable quantification of virtually completely intact rat mtDNA with the use of mouse mtDNA as an internal control or vice versa.\textsuperscript{30} Although different mtDNA lesions may alter long PCR processing and result in lower levels of intact total mtDNA, we believe that this method produces a reasonable index of mtDNA that has maintained sufficient integrity. Briefly, total DNA from mouse CECs or rat brain was purified with the use of genomic DNA isolation kits from Qiagen. DNA was quantified by the PicoGreen method (Molecular Probes). The reaction mixture contained 0.4 ng of mouse total DNA, 4 pmol
of each oligonucleotide primer, 400 μmol/L dNTP mixture, and 0.5 U of LA Taq enzyme (Takara Shuzo Co. Ltd) in a total volume of 10 μL. The same amount (0.4 ng) of total DNA derived from rat brain was added to the PCR reaction mixture as an internal standard. The primer pair for the amplification of 14.3-kb mitochondrial genomes of both rat and mouse were 5′-ATATTTATCAGTCGAGTCCCGTGG-3′ (forward) and 5′-ATTTCGTTGGGTGACCTTTGAG-3′ (reverse). PCR began with denaturation for 1 minute at 94°C, followed by 26 cycles at 94°C for 15 seconds and 68°C for 10 minutes, and a final extension at 72°C for 10 minutes. The PCR products were digested with the restriction enzyme Neol (Promega) at 37°C for 2 hours and fractionated through a 1% agarose gel. The 7.0- and 7.3-kb restriction fragments, representing the amplified mouse mtDNA, do not resolve and migrate as a single band, whereas the 14.3-kb fragment amplified from rat brain mtDNA serves as an internal control. The signal intensities of these bands were assessed by image analysis (Molecular Dynamics) followed by quantitative densitometry with ImageQuant (version 3.3, Molecular Dynamics).

Assessment of Mouse CEC Death
The extent of CEC death was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and LDH assays, as assessed previously.24,26

Caspase-3 and Caspase-8 Activity Assay
Cultured CECs were harvested and then lysed in 200 μL lysis buffer (10 mmol/L Tris–HCl, 1% Triton X-100, 0.32 mol/L sucrose, 5 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride [PMSF], 1 μg/mL aprotinin, 1 μg/mL leupeptin, 2 mmol/L dithiothreitol; pH 8.0). After incubation for 20 minutes on ice, the lysate was spun at 20 000g for 30 minutes at 4°C, and the supernatant was used to detect the activities of the caspases. Cell lysate containing 50 μg protein in 1 mL assay buffer (100 mmol/L HEPES, 10% sucrose, 0.1% CHAPS, 1 mmol/L PMSF, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 2 mmol/L dithiothreitol; pH 7.5) was mixed with the fluorogenic substrate of caspase-3 (Ac-DEVD-7-amino-4-trifluoromethyl-coumarin) or caspase-8 (Ac-IETD-7-amino-4-trifluoromethyl-coumarin) and incubated for 1 hour at 37°C. The fluorescence was measured with a fluorometer adjusted to 400 nm excitation and 505 nm emission with the use of free 7-amino-4-trifluoromethyl-coumarin (Sigma) as a standard (10, 20, 50 nmol/L). Data were presented as percentage of control.

Statistical Analysis
Quantitative data are expressed as mean±SD based on at least 3 separate experiments of triplicate samples. Differences among groups were statistically analyzed by 1-way ANOVA followed by the Bonferroni post hoc test. Comparison between 2 experimental groups was based on 2-tailed t test. A probability value <0.05 was considered significant.

Results
ATM Expression After OGD
OGD caused a rapid increase in ATM mRNA and protein expression in mouse CECs. An increase in ATM mRNA, assessed by RT-PCR, was detected within 1 hour of OGD, persisted up to 8 hours, and was followed by a decline back to baseline levels at 24 hours (Figure 1A). ATM protein assessed by Western blotting showed a corresponding temporal profile, increasing at 2 hours and returning to basal levels between 8 and 24 hours after OGD onset (Figure 1B)

OGD-Induced NF-κB Activation in CECs
OGD also caused an increase in NF-κB DNA binding activity in a time-dependent manner, starting as early as 2 hours after OGD and remaining elevated for at least 6 hours (Figure 2A; a minor nonspecific band is indicated by the asterisk). The specificity of NF-κB DNA binding activity was demonstrated by the complete inhibition of NF-κB binding in the presence of a 100-fold molar excess of cold NF-κB oligonucleotide (data not shown).31 AP-1 DNA binding remained unaltered throughout 24 hours of OGD exposure, suggesting that OGD does not broadly affect all stress-sensitive transcription factors (Figure 2B). To assess the consequences of OGD-induced NF-κB activation on downstream gene expression, we examined the products of genes known to be downstream of NF-κB, including c-IAP2, survivin, and ICAM-1. Immunoblotting showed OGD-induced expression of c-IAP2 for up to 24 hours after OGD in a time-dependent manner that followed NF-κB activation (Figure 2C). OGD did not affect the expression of 2 other genes, survivin and ICAM-1 (Figure 2D and 2E), suggesting selective gene transactivation by OGD-induced NF-κB activation.

Effect of ATM Gene Knockdown on OGD-Induced NF-κB Activation and CEC Death
Given the recent evidence suggesting that NF-κB activation may require ATM activity,32 we investigated this potential link by suppressing ATM gene expression using an antisense oligonucleotide. The success of this gene knockdown approach was demonstrated by the reduction in OGD-induced ATM protein expression (Figure 3A). ATM gene knockdown also attenuated NF-κB DNA activation caused by OGD (Figure 3B). Moreover, the induction of c-IAP2 protein was also suppressed (Figure 3C). The sense oligonucleotide was without effect, confirming the specific action of the antisense oligonucleotide.

NF-κB activation may prevent apoptosis by inducing c-IAP2,3 which binds and inhibits caspase-3 and caspase-8 activation.33,34 Therefore, we examined the effects of ATM gene downregulation on caspase-3 and -8 activity. OGD increased both caspase-3 and -8 activity. ATM knockdown significantly augmented the activity of both caspas (Figure 4A). Our previous work has shown that OGD-induced CEC death occurred in a time-dependent manner, starting as early as 2 hours after OGD and remaining elevated for at least 6 hours (Figure 2A; a minor nonspecific band is indicated by the asterisk). The specificity of NF-κB DNA binding activity was demonstrated by the complete inhibition of NF-κB binding in the presence of a 100-fold molar excess of cold NF-κB oligonucleotide (data not shown).31 AP-1 DNA binding remained unaltered throughout 24 hours of OGD exposure, suggesting that OGD does not broadly affect all stress-sensitive transcription factors (Figure 2B). To assess the consequences of OGD-induced NF-κB activation on downstream gene expression, we examined the products of genes known to be downstream of NF-κB, including c-IAP2, survivin, and ICAM-1. Immunoblotting showed OGD-induced expression of c-IAP2 for up to 24 hours after OGD in a time-dependent manner that followed NF-κB activation (Figure 2C). OGD did not affect the expression of 2 other genes, survivin and ICAM-1 (Figure 2D and 2E), suggesting selective gene transactivation by OGD-induced NF-κB activation.

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death may be associated with extensive DNA fragmentation and apoptosis. Mitochondria may play a key role in the execution of apoptosis. OGD caused mitochondrial dysfunction, as reflected by mtDNA damage in CECs. We further examined the effect of ATM knockdown on the extent of mtDNA using long PCR. OGD resulted in a reduction of the content of mtDNA, suggesting mtDNA damage. ATM knockdown with antisense oligonucleotides aggravated OGD-induced mtDNA damage (Figure 4B) and accentuated OGD-induced cell death (Figure 4C and 4D). Quantification of long PCR data indicates that the decrease in mtDNA content based on the long PCR method was far greater than would be expected by cell death alone: the relative mtDNA content in antisense-treated cells was reduced to 1% of sense-treated controls, while cell survival was reduced to 67% of sense-treated controls (data not shown). These results suggest that OGD-induced ATM expression may confer a cytoprotective action in CECs.

Figure 2. Effects of OGD on NF-κB and AP-1 DNA binding activities and expression of genes downstream of NF-κB. OGD caused a time-dependent elevation of NF-κB DNA binding activity after OGD (A), increasing 2 hours after OGD and returning to basal levels by 8 hours. OGD had little effect on AP-1 DNA binding activity (B). *Nonspecific bands. Immunoblotting showed expression of c-IAP2 (C), survivin (D), and ICAM-1 (E) at various times after OGD initiation. Protein levels of c-IAP2 increased within 4 hours of OGD and persisted up to 24 hours. No significant changes were observed for survivin or ICAM-1. Twenty to 40 μg of cytoplasmic protein per lane was loaded on 10% to 15% SDS-PAGE and processed for Western blotting.

Figure 3. Effects of ATM knockdown on OGD-induced NF-κB activation and downstream gene expression. ATM antisense, but not sense, oligonucleotides reduced ATM protein levels (A), NF-κB DNA binding activity (B), and c-IAP2 protein (C). C indicates vehicle control; S, sense oligonucleotide; and AS, antisense oligonucleotide. Results shown are representative of 3 separate experiments with similar results.
Effect of SN50 on OGD-Induced NF-κB Activation and CEC Death

To confirm that the NF-κB signal transduction pathway is pivotal in OGD-induced CEC death, a specific NF-κB inhibitor, SN50, was used to explore whether suppression of NF-κB activity alters the extent of OGD-induced CEC death. SN50 (30 μg/mL) significantly decreased OGD-induced NF-κB activation and subsequent transactivation of c-IAP2 (Figure 5). SN50 also accentuated OGD-induced caspase-3 and caspase-8 activation and mtDNA damage (Figure 6A and 6B). Finally, NF-κB inhibition by SN50 was accompanied by an increase in OGD-induced CEC death (Figure 6C and 6D). Mitochondrial DNA damage was far greater than would be accounted for by cell death alone. SN50 had no effect on ATM mRNA or protein levels (data not shown). These findings suggest that OGD-induced NF-κB activation is consequential in affecting CEC viability.

Discussion

We used a well-established in vitro ischemic model, OGD in CECs, to address the potential role of ATM in ischemic injury. In this study we found ATM activation after in vitro hypoxic/ischemic insult in CECs: ATM mRNA and protein levels were increased within 4 hours of OGD. We further demonstrated that NF-κB activation was noted subsequent to OGD-induced ATM expression. ATM knockdown by an antisense oligonucleotide significantly reduced NF-κB activation and the expression of its downstream gene, cIAP2. The reduced expression of this antiapoptotic gene was associated with an increase in caspase-3 and caspase-8 activity, mtDNA damage, and cell death. This same ATM antisense oligonucleotide had no effect on survivin or ICAM-1 expression (data not shown), and the ATM sense oligonucleotide was without effect on NF-κB or cell viability, confirming specificity of the antisense oligonucleotide. On the basis of these results, we suggest that OGD induces ATM expression, which, in turn, contributes to the activation of NF-κB in CECs. Activated NF-κB, in turn, transactivates cIAP2, countering caspase activation and cell death.

Our proposal is consistent with the finding that ATM contributes to NF-κB activation by oxidative stress. ATM activates NF-κB by phosphorylating IκB-α, marking it for degradation and thereby relieving its inhibitory influence.

NF-κB transactivates a diverse array of genes, including developmental genes, inflammatory genes such as ICAM-1, and genes involved in the regulation of apoptosis. In some cells, the survival activity of NF-κB is probably mediated by specific downstream target genes, including the inhibitor of apoptosis protein (IAP) genes. Among these NF-κB target genes are c-IAP1, c-IAP2, xIAP, and survivin. Highly conserved between insects and mammals,
IAPs contain at least 1 copy of the characteristic baculovirus IAP repeat (BIR) domain, which bind and directly inhibit caspase-3, -7, and -8.\textsuperscript{33,34} In the present study OGD specifically induced c-IAP2 but not survivin or ICAM-1. Furthermore, ATM knockdown reduced c-IAP2, increased caspase-3 and -8 activities, and accentuated CEC death. Although c-IAP2 is an antiapoptotic protein and its expression is maintained at high levels up to 24 hours after OGD onset, we did not find greater protective activity at the 24-hour time point (data not shown), which suggests that the cytoprotective effect of c-IAP2 was set at an early stage of OGD-induced cell death. This contention is consistent with the findings of others\textsuperscript{40–42} who have demonstrated that early interventions may prevent oxidative damage to DNA and subsequent cell death. Mitochondrial dysfunction has been implicated as a key mechanism in apoptosis in a number of cell death paradigms\textsuperscript{35} and is involved in the initiation of apoptosis by at least 2 different mechanisms: release of mitochondrial intermembrane proteins, resulting in caspase-dependent and caspase-independent apoptotic pathways, and loss of mitochondrial membrane function, leading to failure of mitochondrial potential and energy crisis.\textsuperscript{43} Caspase-8 and -3 activations are upstream and downstream, respectively, of mitochondrial dysfunction in a number of cell death paradigms.\textsuperscript{44} In the present study OGD caused mtDNA damage, consistent with mitochondrial dysfunction, as reflected by activation of caspase-8 (upstream of mitochondria) and caspase-3 (downstream of mitochondria). Knockdown of ATM accentuated mitochondrial dysfunction and related cell death.

That NF-κB activation is consequential in OGD-induced stress in CECs is supported by the effects of SN50, a specific NF-κB inhibitor, which has been shown to block the translocation of NF-κB to the nucleus.\textsuperscript{45,46} Treatment with SN50 significantly reduced OGD-induced NF-κB binding activity, suppressed c-IAP2 expression, and exacerbated CEC death. Evidence of increased mtDNA damage and enhanced caspase-3 and -8 activity was present in cells treated with SN50. Thus, inhibition of NF-κB reproduced the findings of ATM gene knockdown and supported the contention that ATM expression exerts a cytoprotective effect in CECs exposed to OGD via an NF-κB–dependent and antiapoptosis signal transduction pathway. Further investigation is warranted to explore the actions of the ATM gene in endothelial cells in animal models of cerebral ischemia.

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References


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