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RNA interference-mediated silencing of BACE and APP attenuates the isoflurane-induced caspase activation

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Abstract

Background: β-Amyloid protein (Aβ) has been shown to potentiate the caspase-3 activation induced by the commonly used inhalation anesthetic isoflurane. However, it is unknown whether reduction in Aβ levels can attenuate the isoflurane-induced caspase-3 activation. We therefore set out to determine the effects of RNA interference-mediated silencing of amyloid precursor protein (APP) and β-site APP-cleaving enzyme (BACE) on the levels of Aβ and the isoflurane-induced caspase-3 activation.

Methods: H4 human neuroglioma cells stably transfected to express full-length human APP (H4-APP cells) were treated with small interference RNAs (siRNAs) targeted at silencing BACE and APP for 48 hours. The cells were then treated with 2% isoflurane for six hours. The levels of BACE, APP, and caspase-3 were determined using Western blot analysis. Sandwich Enzyme-linked immunosorbent assay (ELISA) was used to determine the extracellular Aβ levels in the conditioned cell culture media.

Results: Here we show for the first time that treatment with BACE and APP siRNAs can decrease levels of BACE, full-length APP, and APP c-terminal fragments. Moreover, the treatment attenuates the Aβ levels and the isoflurane-induced caspase-3 activation. These results further suggest a potential role of Aβ in the isoflurane-induced caspase-3 activation such that the reduction in Aβ levels attenuates the isoflurane-induced caspase-3 activation.

Conclusion: These findings will lead to more studies which aim at illustrating the underlying mechanism by which isoflurane and other anesthetics may affect Alzheimer's disease neuropathogenesis.

Background

Alzheimer's disease (AD), one of the most common forms of dementia, affects 4.5 million Americans and costs more than $100 billion a year on direct care alone. Its impact will only increase in the coming decades. AD is an insidious and progressive neurodegenerative disorder and is characterized by global cognitive decline, robust accumulation of amyloid deposits, and neurofibrillary tangles in the brain [reviewed in [1]]. Genetic evidence, confirmed by neuropathological and biochemical findings, indicates that excessive production and/or accumulation of β-amyloid protein (Aβ) play a fundamental role in the pathology of AD [reviewed by [1,2]]. Aβ is produced from amyloid precursor protein (APP) through proteolytic processing by the aspartyl protease β-site APP-cleaving enzyme (BACE) and γ-secretase [reviewed in [3]].

Increasing evidence suggests a role for caspase activation and apoptosis in AD neuropathogenesis [[4-13], reviewed in [14,15]]. There has been debate in regards to the contribution of apoptosis to neuronal loss in AD because the apoptotic markers are rarely detected in the brain of AD patients [reviewed in [16,17]]. However, this could be due to the long duration of AD and very rapid clearance of apoptotic cells from organs. Recent studies employing antibodies that specifically recognize

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caspase-cleaved substrates have shown that caspase-3-cleaved-actins, caspase-3-cleaved fragments, and caspase-cleaved-APPs are present in AD patients' brains [18-31]. Western blot analysis has also revealed increased caspase-3 immunoreactivity in AD versus control brains [24,32,33]. In addition, activated caspase-6 and caspase-9 have been detected in AD brains [25,26].

An estimated 200 million patients worldwide undergo anesthesia and surgery each year [34,35]. Both surgery and anesthesia have been suggested to play a role in the progress of AD neuropathogenesis [reviewed in [36,37]] and AD. Specifically, the age of onset of AD has been reported to be inversely related to cumulative exposure to anesthesia and surgery before the age of 50 years [38], even though anesthesia and/or surgery themselves may not increase the incidence of AD [39]. Another study showed that patients having coronary artery bypass graft surgery under general anesthesia may be at increased risk for AD as compared to those having percutaneous transluminal coronary angioplasty under local anesthesia [40]. A recent retrospective population-based study has found that general anesthesia is a risk factor of AD with an adjusted odds ratio of 3.22 [41]. Moreover, cognitive dysfunction or decline occurs after anesthesia and surgery [[42-52], reviewed in 53], which is associated with impairments in daily functioning [54], dependency on government economic assistance [52], and increased morbidity and mortality [[42,55], reviewed in [56]]. However, opposing findings also exist [57-59]. Therefore, more clinical studies, which will define the role of anesthesia and/or surgery in AD and in postoperative cognitive dysfunction or decline, are necessary [60].

Given the fact that adequately powered prospective human studies will take many years to conduct and analyze, it is equally important to perform animal and *in vitro* studies, which will complement ongoing human studies, e.g., by establishing a mechanistic hypothesis. Several studies have shown that the commonly used inhalation anesthetic isoflurane may induce caspase activation, apoptosis, Aβ oligomerization and accumulation, neuroinflammation, tau protein hyperphosphorylation, mitochondrial dysfunction, and impairment of learning and memory [[60-69], reviewed in [36,37]]. However, the underlying mechanisms of these effects remain largely to be determined. Our studies in cultured cells have shown that exogenerously administrated Aβ into the cell culture media can potentiate the isoflurane-induced caspase activation and apoptosis, which may induce further rounds of apoptosis and Aβ generation [70]. In the present studies, we set out to determine the effects of RNA interference (RNAi)-mediated silencing of BACE and APP on Aβ levels and the isoflurane-induced caspase activaion in cultured cells to further elucidate the potential association of Aβ accumulation and the isoflurane-induced caspase-3 activation.

Methods

Cell lines

We employed H4 human neuroglioma cells stably transfected to express full-length human APP (H4-APP cells) in the experiments. We used H4-APP cells for the easy measurement of Aβ levels in the conditioned cell culture media as we did in the previous studies [65,70,71]. The cells were cultured in Dulbecco's modified Eagle's medium (high glucose) containing 9% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 g/ml streptomycin, and 2 mM L-glutamine and was supplemented with 20 g/ml G418.

RNAi studies

RNAi-mediated silencing of BACE and APP experiments were similar to those in our previous studies [72-76]. In order to avoid off-target effects of RNAi, we employed two sets of small interference RNAs (siRNAs) aimed at silencing of BACE (1^{st} set: 3'GCAAGGAGUACAACUAUGAUU; 2^{nd} set: 3'GGAGGGAGCAUGAUCAUUGUU) and APP (1^{st} set: 3' GGUGGGCGGUGUUGUCAUA; 2^{nd} set: 3' GGUUCUGGGUUGACAAAUA). These siRNAs and control siRNA (3'UAGCGACUAAACACAUCAAUU) were obtained from Dharmacon (Lafayette, CO). siRNAs were transfected into cells using electroporation (AMAXA, Gaithersburg, MD) as described by Xie et al [75]. Briefly, we mixed 1 million cells, 100 ul AMAXA electroporation transfection solution and 10 ul 20 uM siRNA together, then we employed C-9 program in an AMAXA electroporation device for cell transfection. The transfected cells were then placed in one of the six-well plates containing 1.5 ml cell culture media. The BACE, APP, or control siRNA-pretreated cells were then exposed to the isoflurane treatment 48 hours later.

Isoflurane treatment

The isoflurane treatment was similar to those in our previous studies [65,70,71]. We chose 2% isoflurane (air component: 2% isoflurane, 5% CO_2, 21% O_2 and balanced nitrogen) in the studies based on our previous studies [65,70,71]. The control condition included 5% CO_2 plus 21% O_2 (air component: 5% CO_2, 21% O_2 and balanced nitrogen), which did not affect caspase-3 activation or Aβ levels (Data not shown). The delivery of gases was similar to that described in our previous studies [65,70]. Briefly, 21% O_2, 5% CO_2, and 2% isoflurane were delivered from an anesthesia machine to a sealed plastic box (airtight chamber) in a 37 degree C incubator containing six-well plates seeded with one million cells in a 1.5 ml cell culture media. The Datex infrared gas analyzer (Puritan-Bennett, Tewksbury, MA) was

used to continuously monitor the concentrations of CO_2, O_2, and isoflurane that were delivered.

Lysis of cells and protein amount quantification

The pellets of the cells were detergent-extracted on ice using an immunoprecipitation buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 0.5% Nonidet P-40) plus protease inhibitors (1 g/ml aprotinin, 1 g/ml leupeptin, 1 g/ml pepstatin A). The lysates were collected and centrifuged at 12,000 × g for 10 minutes, and then were quantified for total protein levels using the bicinchoninic acid protein assay kit (Pierce, Iselin, NJ).

Western blot analysis

The cells were harvested at the end of the experiments and were subjected to Western blot analyses using the methods described by Xie et al. [70]. BACE antibody (1:1,000 dilution; Abcam, Cambridge, MA) was used to recognize BACE (65 kDa). Antibody A8717 (1:1,000 dilution; Sigma, St. Louis, MO) was used to recognize FL-APP (110 kDa) and APP-CTFs (10 to 12 kDa). A caspase-3 antibody (1:1,000 dilution; Cell Signaling Technology, Inc. Beverly, MA) was used to recognize the caspase-3 fragment (17-20 kDa), which results from cleavage at the asparate position 175, and FL-caspase-3 (35 - 40 kDa). An antibody to the non-targeted protein β-Actin (42 kDa, 1:5,000, Sigma) was used to control for loading differences in total protein amounts. Each band in the Western blot represents an independent experiment. We have averaged the results from three to six independent experiments. The intensity of signals in each Western blot was analyzed using the National Institute of Health image program (National Institute of Health Image 1.62, Bethesda, MD). We quantified the Western blots using two steps. First, we used levels of β-Actin to normalize (e.g., determine the ratio of the amount of FL-caspase-3 to the amount of β-Actin) the levels of FL-caspase-3, caspase-3 fragment, BACE, FL-APP, and APP-CTFs to control for any loading differences in total protein amounts. Second, we presented changes in the levels of BACE, FL-APP, APP-CTFs, and caspase-3 in the treated cells as percentages of those in cells from the control condition.

Quantification of Aβ using Sandwich ELISA assay

Secreted Aβ in the conditioned culture media was measured with a Sandwich ELISA assay by using an Aβ measurement kit (Invitrogen, Carlsbad, CA) as described by Xie et al. [75]. Specifically, 96-well plates were coated with mouse monoclonal antibodies (mAb) specific to $Aβ_{40}$ (2G3) or $Aβ_{42}$ (21F12). Following blocking with Block Ace, wells were incubated overnight at 4°C with test samples of conditioned cell culture media, and then an anti-Aβ (β-A-HR1) antibody conjugated to horseradish peroxidase was added. Plates were then developed with TMB reagent and well absorbance was measured at 450 nm. Aβ levels in test samples were determined by comparison with the signal from unconditioned media spiked with known quantities of $Aβ_{40}$ and $Aβ_{42}$.

Statistics

Given the presence of background caspase-3 activation, Aβ, BACE, FL-APP, and APP-CTFs in the cells cultured in serum free media, we did not use absolute values to describe their changes. Instead, these changes were presented as percentages of those from the control group. For example, one hundred percent of caspase-3 activation refers to the control level for the purpose of comparison to experimental conditions. Data were expressed as mean ± S.D.. The number of samples varied from three to six, and the samples were normally distributed. We used a two-tailed t-test to compare the difference between the control siRNA and BACE or APP siRNA, and the control condition and isoflurane treatment. P-values less than 0.05 (*) and 0.01 (** or ##) were considered statistically significant.

Results and discussion

RNAi-mediated silencing of BACE attenuates the isoflurane-induced caspase-3 activation

We previously reported that the commonly used inhalation anesthetic isoflurane can induce caspase activation and apoptosis *in vitro* [65,70,71] and *in vivo* [64]. However, the underlying mechanisms of these effects remain largely to be determined. Specifically, Aβ has been shown to potentiate the isoflurane-induced caspase-3 activation in H4 naïve cells, but it is largely unknown whether reduction in the levels of Aβ can decrease the isoflurane-induced caspase-3 activation in the cultured cells. BACE is the enzyme for Aβ generation and APP is the precursor of Aβ. Decreases in the levels of BACE and APP could lead to reduction in Aβ levels [3]. We therefore set out to assess the effects of RNAi-mediated silencing of BACE and APP on the levels of Aβ and the isoflurane-induced caspase-3 activation in H4-APP cells.

The H4-APP cells were treated with control or BACE siRNA for 48 hours before the treatment with 2% isoflurane for six hours. The cells were harvested at the end of the experiment and were subjected to Western blot analysis. BACE immunoblotting showed that the BACE siRNA treatment decreased BACE levels as compared to the control siRNA treatment (Figure 1A). The quantification of the Western blots illustrated that BACE siRNA treatment significantly decreased BACE levels as compared to control siRNA: 100% versus 57% (Figure 1B). These findings suggest that the treatment with BACE siRNA, which targets at reducing mRNA

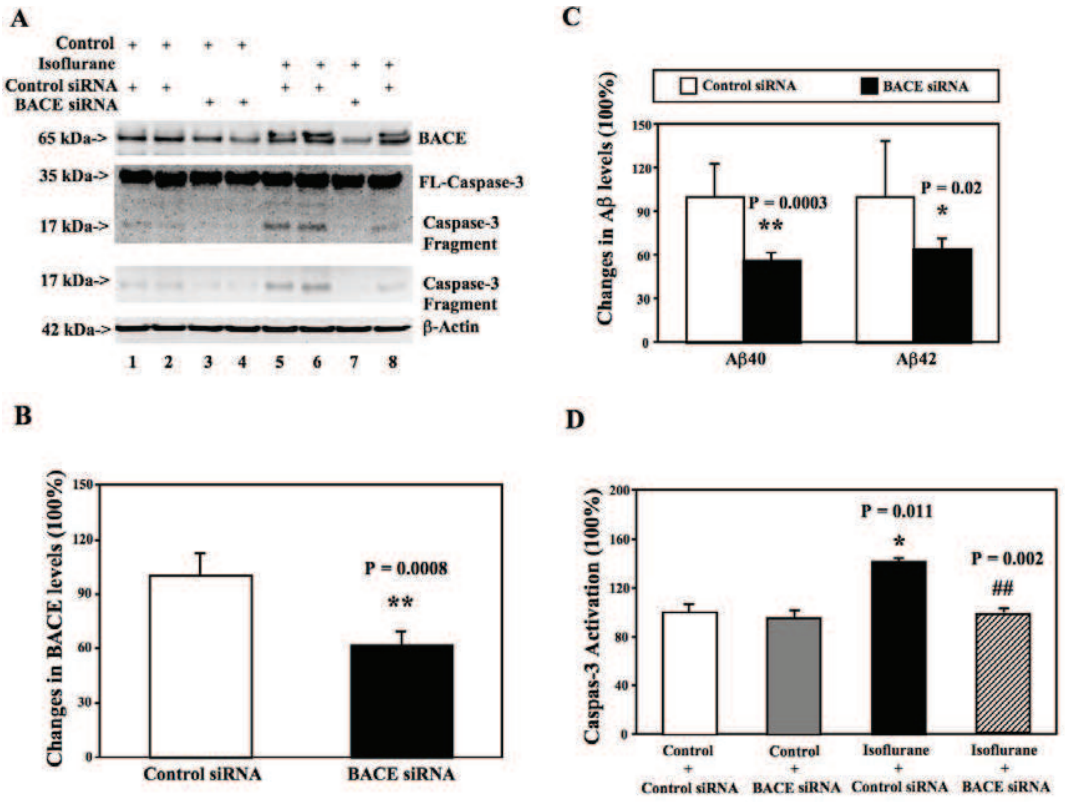

Figure 1 Effects of RNAi-mediated silencing of BACE on Aβ levels and caspase-3 activation in H4-APP cells. **A**. Treatment of BACE siRNA (lanes 3, 4 and 7) decreases the levels of BACE as compared to control siRNA (lanes 1, 2, 5, 6 and 8) in the Western blotting analysis. Isoflurane treatment (lanes 5 and 6) induces caspase-3 activation as compared to control condition (lanes 1 and 2). The BACE siRNA treatment alone (lanes 3 and 4) does not induce caspase-3 activation. However, the BACE siRNA treatment (lane 7) attenuates the isoflurane-induced caspase-3 activation (lanes 5, 6 and 8) as compared to control siRNA treatment (lanes 5, 6 and 8). **B**. Quantification of the Western blots shows that BACE siRNA treatment (black bar) decreases the levels of BACE (** P = 0.0008) as compared to control siRNA treatment (white bar). **C**. BACE siRNA treatment (black bar) reduces the levels of Aβ40 (left panel, ** P = 0.0003) and Aβ42 (right panel, * P = 0.02) as compared to control siRNA treatment (white bar). **D**. Quantification of the Western blots shows that isoflurane (black bar, * P = 0.011) induces caspase-3 activation as compared to control condition (white bar). BACE siRNA treatment (net bar, ## P = 0.002) attenuates the isoflurane-induced caspase-3 activation as compared to control siRNA plus isoflurane treatment (black bar).

levels of BACE, was able to reduce the protein levels of BACE in the current experiment. Next, we were able to show that the BACE siRNA treatment decreased the levels of both Aβ40 (100% versus 55%) and Aβ42 (100% versus 63%) (Figure 1C). These results suggested that the BACE siRNA was able to reduce Aβ generation by decreasing the levels of BACE, the enzyme of Aβ generation.

As expected, the caspase-3 immunoblotting showed that the treatment with 2% isoflurane (lanes 5, 6 and 8) for six hours induced caspase-3 activation, as evidenced by increased ratios of cleaved (activated) caspase-3 fragment (17 kDa) to full-length (FL) caspase-3 (35 - 40 kDa), compared with control condition (lanes 1 and 2). Finally, we were able to show that the BACE siRNA treatment (lane 7) attenuated the isoflurane-induced caspase-3 activation (lanes 5, 6 and 8) (Figure 1A). The quantification of the Western blots showed that the isoflurane treatment (black bar) induced caspase-3 activation as compared to control condition (white bar): 100% versus 148%. The BACE siRNA treatment alone (gray bar) did not induce caspase activation. However, the BACE siRNA treatment attenuated the isoflurane-induced caspase-3 activation (net bar) (Figure 1D): 148% versus 103%. These results illustrate that reduction in BACE levels, via RNAi-mediated silencing of BACE, may lead to the reduction of Aβ levels and the attenuation of the isoflurane-induced caspase-3 activation.

RNAi-mediated silencing of APP attenuates the isoflurane-induced caspase-3 activation

Given the findings that reduction in the levels of both BACE and Aβ is associated with the attenuation of the isoflurane-induced caspase-3 activation, next, we would like to know whether other methods to reduce Aβ levels

can also lead to the attenuation of the isoflurane-induced caspase-3 activation. Therefore, we set out to determine the effects of RNAi-mediated silencing of APP, the precursor of Aβ, on the levels of APP and Aβ, and on the isoflurane-induced caspase-3 activation.

The H4-APP cells were treated with control or APP siRNA for 48 hours before the treatment with 2% isoflurane for six hours. The cells were harvested at the end of the experiment and were subjected to Western blot analysis. The APP immunoblotting showed that the APP siRNA treatment (lanes 3 and 4) decreased the levels of FL-APP and APP-CTFs as compared to the control siRNA treatment (lanes 1 and 2) (Figure 2A). The quantification of the Western blots showed that the APP siRNA treatment (black bar) decreased the levels of FL-APP (left panel, 100% versus 26%) and APP-CTFs (right panel, 100% versus 23%) as compared to control siRNA treatment (white bar). These results suggest that the RNAi-mediated silencing of APP was able to reduce the levels of APP in the H4-APP cells in the current experiment.

Next, we were able to show that the APP siRNA treatment reduced the levels of both Aβ40 (left panel, 100% versus 58%) and Aβ42 (right panel, 100% versus 66%). Finally, the caspase-3 immunoblotting showed that the APP siRNA treatment (lanes 3 and 4) decreased the isoflurane-induced caspase-3 activation as compared to the control siRNA treatment (lanes 1 and 2) (Figure 2D). The quantification of the Western blots showed that the APP siRNA treatment (black bar) decreased the isoflurane-induced caspase-3 activation as compared to control siRNA treatment (white bar): 100% versus 64%. These results illustrated that the reduction in the levels of Aβ and APP, resulting from RNAi-mediated silencing of APP, may also lead to the attenuation of isoflurane-induced caspase-3 activation.

Taken together, these findings suggest that there is an association between the Aβ levels and the isoflurane-induced caspase-3 activation, specifically, the reduction of Aβ levels, resulted from RNAi-mediated silencing of either BACE or APP, can lead to the attenuation of the isoflurane-induced caspase-3 activation.

Our previous studies have shown that the commonly used inhalation anesthetic isoflurane can induce caspase-3 activation and apoptosis [64,65,70,71]. However, the underlying mechanism remains unclear and is an important question in the field of anesthesia neurotoxicity research. The previous studies in H4 naïve and H4-APP cells have shown that the isoflurane-induced caspase-3 activation and apoptosis can enhance levels of BACE and γ-secretase, which promote APP processing and increase Aβ generation [70]. Moreover, Aβ can potentiate the isoflurane-induced caspase-3 activation, leading to further rounds of apoptosis [70]. However, it is largely unknown whether reduction in Aβ levels can attenuate the isoflurane-induced caspase-3 activation. Therefore, we set out to assess the effects of RNAi-mediated silencing of APP, the precursor of Aβ, and BACE, the enzyme of Aβ generation, on Aβ levels and on the isoflurane-induced caspase-3 activation in H4-APP cells.

First, we have found that RNAi-mediated silencing of BACE can decrease BACE levels. These results suggest that the BACE siRNA-induced reduction in BACE mRNA levels can successfully decrease the protein levels of BACE in the current experiment. Then, we have found that there is a decrease in Aβ levels following the BACE siRNA treatment. Finally, the BACE siRNA treatment attenuates the isoflurane-induced caspase-3 activation in the H4-APP cells. These results have suggested that decreased Aβ levels by the RNAi-mediated silencing of BACE may lead to the attenuation of the isoflurane-induced caspase-3 activation. These results further support our previous findings that isoflurane may induce a vicious cycle of caspase-3 activation/apoptosis and Aβ accumulation [70].

The double bands for BACE in Figure 1A could be the isoforms of BACE. It is also possible that isoflurane induces a post-translational modification of BACE (e.g., phosphorylation). However, the RNAi of BACE decreases both bands of BACE, thus these findings still support the conclusion of current study that RNAi-mediated silencing of BACE can lead to a reduction in Aβ levels and an attenuation of the isoflurane-induced caspase-3 activation. As the key enzyme that initiates the formation of Aβ, BACE is a prerequisite for the generation of Aβ, which gives rise to cerebrovascular and parenchymal amyloid plaque in the brain of AD patients. Thus, it is important to identify these double bands following the isoflurane treatment in the future studies.

Previous in vivo studies have shown that a 50% reduction in BACE1 levels causes only a 12% decrease in Aβ levels in heterozygous BACE1 gene knock-out mice [77]. However, our current in vitro studies have illustrated that a 43% reduction in BACE levels, following the BACE siRNA treatment, led to a 45% and a 37% reduction in the levels of Aβ40 and Aβ42, respectively. It is largely unknown why there is a difference between the in vitro and in vivo findings in the Aβ levels. The possible explanations include the difference in the methods and experimental variability.

Decreased levels of BACE in heterozygous (BACE1 +/-) mice can lead to improvement of hippocampus-independent and -dependent form of memory deficits in the AD animal model [78,79]. Isoflurane has been shown to induce learning and memory impairment [62,80,81]. Our future studies, therefore, will include

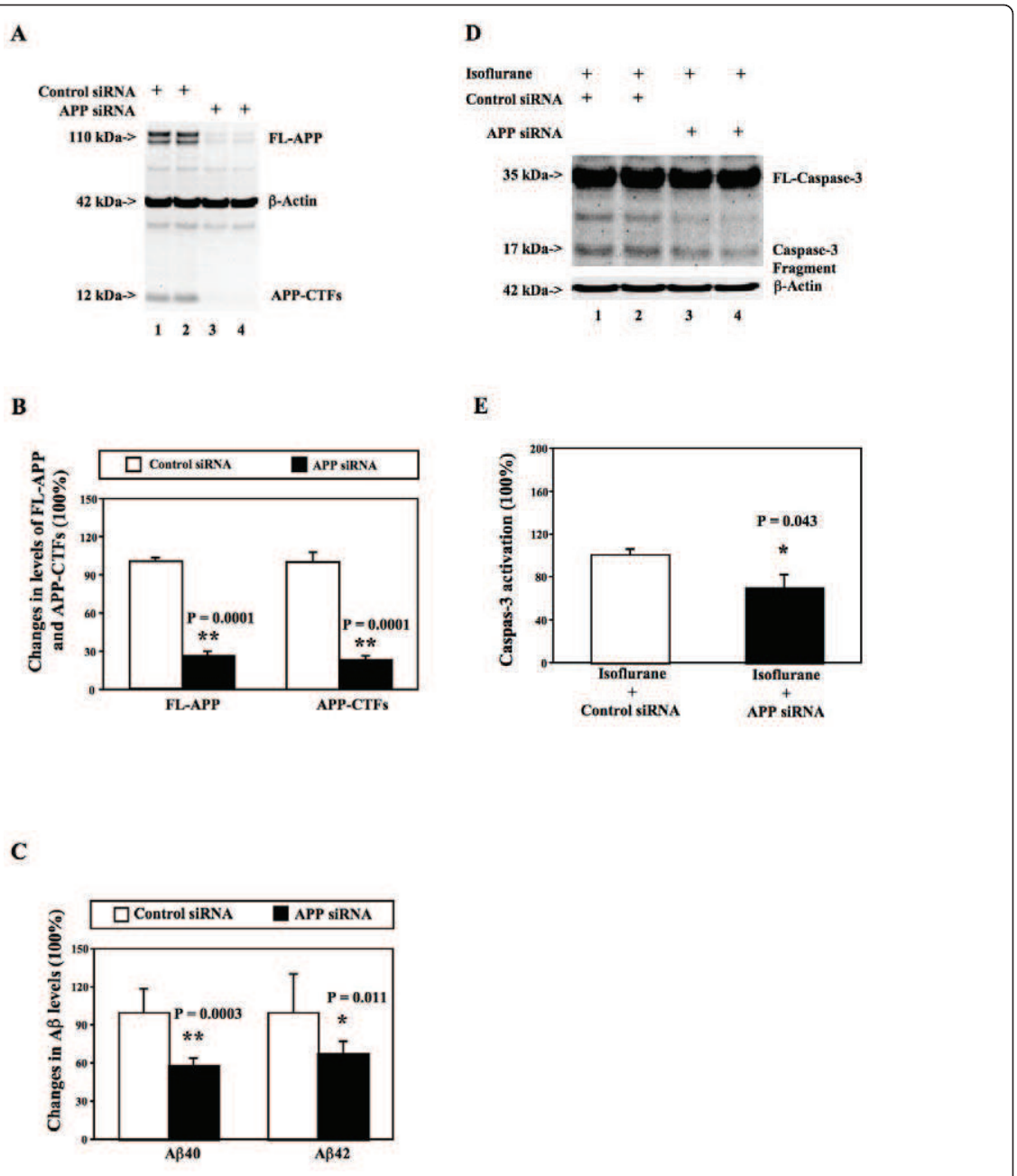

Figure 2 Effects of RNAi-mediated silencing of APP on Aβ levels and caspase-3 activation in H4-APP cells. **A**. Treatment of APP siRNA (lanes 3 and 4) decreases the levels of FL-APP and APP-CTFs as compared to control siRNA (lanes 1 and 2) in the Western blotting analysis. **B**. Quantification of the Western blots shows that APP siRNA treatment (black bar) decreases the levels of FL-APP (left panel, ** P = 0.0001) and APP-CTFs (right panel, ** P = 0.0001) as compared to control siRNA treatment (white bar). **C**. APP siRNA treatment (black bar) reduces the levels of Aβ40 (left panel, ** P = 0.0003) and Aβ42 (right panel, * P = 0.011) as compared to control siRNA treatment (white bar). **D**. APP siRNA treatment (lanes 3 and 4) attenuates the caspase-3 activation induced by isoflurane as compared to the control siRNA treatment (lanes 1 and 2) in the Western blotting analysis. **E**. Quantification of the Western blots shows that APP siRNA treatment (black bar, * P = 0.043) reduces the isoflurane-induced caspase-3 activation as compared to control siRNA plus isoflurane treatment (white bar).

assessing the effects of isoflurane on learning and memory in heterozygous (BACE1+/-) mice to further determine the role of BACE and Aβ in the anesthesia associated neurotoxicity.

Next, we have further demonstrated the potential association of Aβ accumulation and isoflurane-induced caspase-3 activation by showing that RNAi-mediated silencing of APP can decrease the levels of FL-APP,

APP-CTFs, Aβ, and finally the isoflurane-induced caspase-3 activation. These findings have suggested that the reduction in Aβ levels by decreasing the levels of its precursor i.e., APP, can also lead to the attenuation in the isoflurane-induced caspase-3 activation.

Isoflurane has been reported to induce caspase activation and apoptosis [64,65,70,76,82], [reviewed in [36,37]]. However, different findings do exist [83-93]. The reason for the different effects of isoflurane is largely unknown. Some studies have suggested that isoflurane may have a concentration and/or time-dependent dual effect (protective versus toxic) [94-96]. However, given the findings that increases and decreases in Aβ levels can either potentiate [70] or attenuate (current findings) the isoflurane-induced caspase-3 activation, respectively, it is possible that isoflurane may have different effects on caspase-3 activation and apoptosis when different Aβ levels are presented. Additional studies will be needed to further test this hypothesis by determining the effects of different concentrations of exogenously administrated Aβ on the isoflurane-induced caspase-3 activation and apoptosis *in vitro* and *in vivo*.

Conclusion

In conclusion, we have found that RNAi-mediated silencing of either BACE or APP can lead to a reduction in Aβ levels as well as an attenuation in the isoflurane-induced caspase-3 activation. These results have further supported our previous findings that isoflurane induces caspase activation and apoptosis, which lead to Aβ accumulation. Aβ will then cause further rounds of caspase activation and apoptosis [70]. We would like to emphasize that although our current findings and the results from other studies have suggested that isoflurane may promote AD neuropathogenesis, it is still premature to conclude that isoflurane is toxic to use in patients. The *in vivo* relevance of these effects of isoflurane in humans remains largely to be determined. Nevertheless, our current findings should lead to additional studies to determine the potential effects of anesthetics on AD neuropathogenesis and the underlying mechanisms. These efforts will ultimately help facilitating the design of safer anesthetics and improved anesthesia care for patients, especially elderly individuals and patients with AD.

List of Abbreviation

AD: Alzheimer's disease; APP: amyloid β precursor protein; BACE: β-site amyloid precursor protein-cleaving enzyme; Aβ: β-amyloid protein; CTFs: c-terminal fragments.

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Authors' contributions

YD: Acquisition of data. ZX: Acquisition of data, Analysis and interpretation of data, Critical revision of the manuscript for important intellectual content. YZ: Acquisition of data, Critical revision of the manuscript for important intellectual content. SM: Critical revision of the manuscript for important intellectual content. HW: Administrative, technical, and material support. X S: Administrative, technical, and material support. YY: Obtained funding, Critical revision of the manuscript for important intellectual content. ZX: Obtained funding, Study concept and design, Analysis and interpretation of data, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content, Study supervision. All authors read and have approved the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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References

1. Tanzi RE, Bertram L: **Alzheimer's disease: The latest suspect.** *Nature* 2008, **454**:706-8.
2. Selkoe DJ: **Alzheimer's disease: genes, proteins, and therapy.** *Physiol Rev* 2001, **81**:741-66.
3. Xie Z, Tanzi RE: **Alzheimer's disease and post-operative cognitive dysfunction.** *Exp Gerontol* 2006, **41**:346-59.
4. Holtzman DM, Deshmukh M: **Caspases: a treatment target for neurodegenerative disease?** *Nat Med* 1997, **3**:954-5.
5. Lunkes A, Trottier Y, Mandel JL: **Pathological mechanisms in Huntington's disease and other polyglutamine expansion diseases.** *Essays Biochem* 1998, **33**:149-63.
6. Namura S, Zhu J, Fink K, *et al*: **Activation and cleavage of caspase-3 in apoptosis induced by experimental cerebral ischemia.** *J Neurosci* 1998, **18**:3659-68.
7. Kim TW, Pettingell WH, Jung YK, Kovacs DM, Tanzi RE: **Alternative cleavage of Alzheimer-associated presenilins during apoptosis by a caspase-3 family protease.** *Science* 1997, **277**:373-6.
8. Loetscher H, Deuschle U, Brockhaus M, *et al*: **Presenilins are processed by caspase-type proteases.** *J Biol Chem* 1997, **272**:20655-9.
9. Barnes NY, Li L, Yoshikawa K, Schwartz LM, Oppenheim RW, Milligan CE: **Increased production of amyloid precursor protein provides a substrate for caspase-3 in dying motoneurons.** *J Neurosci* 1998, **18**:5869-80.
10. Kovacs DM, Mancini R, Henderson J, *et al*: **Staurosporine-induced activation of caspase-3 is potentiated by presenilin 1 familial Alzheimer's disease mutations in human neuroglioma cells.** *J Neurochem* 1999, **73**:2278-85.

11. Su JH, Anderson AJ, Cummings BJ, Cotman CW: **Immunohistochemical evidence for apoptosis in Alzheimer's disease.** *Neuroreport* 1994, **5**:2529-33.
12. Su JH, Deng G, Cotman CW: **Bax protein expression is increased in Alzheimer's brain: correlations with DNA damage, Bcl-2 expression, and brain pathology.** *J Neuropathol Exp Neurol* 1997, **56**:86-93.
13. Tesco G, Koh YH, Kang EL, *et al*: **Depletion of GGA3 stabilizes BACE and enhances beta-secretase activity.** *Neuron* 2007, **54**:721-37.
14. Mattson MP: **Contributions of mitochondrial alterations, resulting from bad genes and a hostile environment, to the pathogenesis of Alzheimer's disease.** *Int Rev Neurobiol* 2002, **53**:387-409.
15. Raina AK, Hochman A, Ickes H, *et al*: **Apoptotic promoters and inhibitors in Alzheimer's disease: Who wins out?** *Prog Neuropsychopharmacol Biol Psychiatry* 2003, **27**:251-4.
16. LeBlanc AC: **The role of apoptotic pathways in Alzheimer's disease neurodegeneration and cell death.** *Curr Alzheimer Res* 2005, **2**:389-402.
17. Cribbs DH, Poon WW, Rissman RA, Blurton-Jones M: **Caspase-mediated degeneration in Alzheimer's disease.** *Am J Pathol* 2004, **165**:353-5.
18. Rohn TT, Rissman RA, Head E, Cotman CW: **Caspase Activation in the Alzheimer's Disease Brain: Tortuous and Torturous.** *Drug News Perspect* 2002, **15**:549-57.
19. Rohn TT, Rissman RA, Davis MC, Kim YE, Cotman CW, Head E: **Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain.** *Neurobiol Dis* 2002, **11**:341-54.
20. Rohn TT, Head E, Su JH, *et al*: **Correlation between caspase activation and neurofibrillary tangle formation in Alzheimer's disease.** *Am J Pathol* 2001, **158**:189-98.
21. Rohn TT, Head E, Nesse WH, Cotman CW, Cribbs DH: **Activation of caspase-8 in the Alzheimer's disease brain.** *Neurobiol Dis* 2001, **8**:1006-16.
22. Pompl PN, Yemul S, Xiang Z, *et al*: **Caspase gene expression in the brain as a function of the clinical progression of Alzheimer disease.** *Arch Neurol* 2003, **60**:369-76.
23. Yang F, Sun X, Beech W, *et al*: **Antibody to caspase-cleaved actin detects apoptosis in differentiated neuroblastoma and plaque-associated neurons and microglia in Alzheimer's disease.** *Am J Pathol* 1998, **152**:379-89.
24. Gervais FG, Xu D, Robertson GS, *et al*: **Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic A beta peptide formation.** *Cell* 1999, **97**:395-406.
25. LeBlanc A, Liu H, Goodyer C, Bergeron C, Hammond J: **Caspase-6 role in apoptosis of human neurons, amyloidogenesis, and Alzheimer's disease.** *J Biol Chem* 1999, **274**:23426-36.
26. Lu DC, Rabizadeh S, Chandra S, *et al*: **A second cytotoxic proteolytic peptide derived from amyloid beta-protein precursor.** *Nat Med* 2000, **6**:397-404.
27. Eckert A, Marques CA, Keil U, Schussel K, Muller WE: **Increased apoptotic cell death in sporadic and genetic Alzheimer's disease.** *Ann N Y Acad Sci* 2003, **1010**:604-9.
28. Zhao M, Su J, Head E, Cotman CW: **Accumulation of caspase cleaved amyloid precursor protein represents an early neurodegenerative event in aging and in Alzheimer's disease.** *Neurobiol Dis* 2003, **14**:391-403.
29. Gastard MC, Troncoso JC, Koliatsos VE: **Caspase activation in the limbic cortex of subjects with early Alzheimer's disease.** *Ann Neurol* 2003, **54**:393-8.
30. Hitomi J, Katayama T, Eguchi Y, *et al*: **Involvement of caspase-4 in endoplasmic reticulum stress-induced apoptosis and Abeta-induced cell death.** *J Cell Biol* 2004, **165**:347-56.
31. Takuma H, Tomiyama T, Kuida K, Mori H: **Amyloid beta peptide-induced cerebral neuronal loss is mediated by caspase-3 in vivo.** *J Neuropathol Exp Neurol* 2004, **63**:255-61.
32. Shimohama S, Tanino H, Fujimoto S: **Changes in caspase expression in Alzheimer's disease: comparison with development and aging.** *Biochem Biophys Res Commun* 1999, **256**:381-4.
33. Masliah E, Mallory M, Alford M, Tanaka S, Hansen LA: **Caspase dependent DNA fragmentation might be associated with excitotoxicity in Alzheimer disease.** *J Neuropathol Exp Neurol* 1998, **57**:1041-52.
34. Moonesinghe SR, Mythen MG, Grocott MP: **Review article: high-risk surgery: epidemiology and outcomes.** *Anesth Analg* 2011, **112**:891-901.
35. Weiser TG, Regenbogen SE, Thompson KD, *et al*: **An estimation of the global volume of surgery: a modelling strategy based on available data.** *Lancet* 2008, **372**:139-44.
36. Tang J, Eckenhoff MF, Eckenhoff RG: **Anesthesia and the old brain.** *Anesth Analg* 2010, **110**:421-6.
37. Bittner EA, Yue Y, Xie Z: **Brief review: Anesthetic neurotoxicity in the elderly, cognitive dysfunction and Alzheimer's disease.** *Can J Anaesth* 2011, **58(2)**:216-23.
38. Bohnen N, Warner MA, Kokmen E, Kurland LT: **Early and midlife exposure to anesthesia and age of onset of Alzheimer's disease.** *Int J Neurosci* 1994, **77**:181-5.
39. Bohnen NI, Warner MA, Kokmen E, Beard CM, Kurland LT: **Alzheimer's disease and cumulative exposure to anesthesia: a case-control study.** *J Am Geriatr Soc* 1994, **42**:198-201.
40. Lee TA, Wolozin B, Weiss KB, Bednar MM: **Assessment of the emergence of Alzheimer's disease following coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty.** *J Alzheimers Dis* 2005, **7**:319-24.
41. Bufill E, Bartes A, Moral A, *et al*: **[Genetic and environmental factors that may influence in the senile form of Alzheimer's disease: nested case control studies].** *Neurologia* 2009, **24**:108-12.
42. Monk TG, Weldon BC, Garvan CW, *et al*: **Predictors of cognitive dysfunction after major noncardiac surgery.** *Anesthesiology* 2008, **108**:18-30.
43. Price CC, Garvan CW, Monk TG: **Type and severity of cognitive decline in older adults after noncardiac surgery.** *Anesthesiology* 2008, **108**:8-17.
44. Moller JT, Cluitmans P, Rasmussen LS, *et al*: **Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. International Study of Post-Operative Cognitive Dysfunction.** *Lancet* 1998, **351**:857-61.
45. Newman MF, Kirchner JL, Phillips-Bute B, *et al*: **Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery.** *N Engl J Med* 2001, **344**:395-402.
46. Koch S, Forteza A, Lavernia C, *et al*: **Cerebral fat microembolism and cognitive decline after hip and knee replacement.** *Stroke* 2007, **38**:1079-81.
47. Rodriguez RA, Tellier A, Grabowski J, *et al*: **Cognitive dysfunction after total knee arthroplasty: effects of intraoperative cerebral embolization and postoperative complications.** *J Arthroplasty* 2005, **20**:763-71.
48. Bitsch M, Foss N, Kristensen B, Kehlet H: **Pathogenesis of and management strategies for postoperative delirium after hip fracture: a review.** *Acta Orthop Scand* 2004, **75**:378-89.
49. Bekker AY, Weeks EJ: **Cognitive function after anaesthesia in the elderly.** *Best Pract Res Clin Anaesthesiol* 2003, **17**:259-72.
50. Amador LF, Goodwin JS: **Postoperative delirium in the older patient.** *J Am Coll Surg* 2005, **200**:767-73.
51. McDonagh DL, Mathew JP, White WD, *et al*: **Cognitive function after major noncardiac surgery, apolipoprotein E4 genotype, and biomarkers of brain injury.** *Anesthesiology* 2010, **112**:852-9.
52. Steinmetz J, Christensen KB, Lund T, Lohse N, Rasmussen LS: **Long-term consequences of postoperative cognitive dysfunction.** *Anesthesiology* 2009, **110**:548-55.
53. Sanders RD, Maze M: **Neuroinflammation and postoperative cognitive dysfunction: can anaesthesia be therapeutic?** *Eur J Anaesthesiol* 2010, **27**:3-5.
54. Phillips-Bute B, Mathew JP, Blumenthal JA, *et al*: **Association of neurocognitive function and quality of life 1 year after coronary artery bypass graft (CABG) surgery.** *Psychosom Med* 2006, **68**:369-75.
55. Deiner S, Silverstein JH: **Postoperative delirium and cognitive dysfunction.** *Br J Anaesth* 2009, **103(Suppl 1)**:i41-6.
56. Tsai TL, Sands LP, Leung JM: **An Update on Postoperative Cognitive Dysfunction.** *Adv Anesth* 2010, **28**:269-84.
57. Knopman DS, Petersen RC, Cha RH, Edland SD, Rocca WA: **Coronary artery bypass grafting is not a risk factor for dementia or Alzheimer disease.** *Neurology* 2005, **65**:986-90.
58. Gasparini M, Vanacore N, Schiaffini C, *et al*: **A case-control study on Alzheimer's disease and exposure to anesthesia.** *Neurol Sci* 2002, **23**:11-4.
59. Avidan MS, Searleman AC, Storandt M, *et al*: **Long-term cognitive decline in older subjects was not attributable to noncardiac surgery or major illness.** *Anesthesiology* 2009, **111**:964-70.
60. Harris RA, Eger EI: **Alzheimer's disease and anesthesia: out of body, out of mind...or not?** *Ann Neurol* 2008, **64**:595-7.
61. Culley DJ, Baxter MG, Crosby CA, Yukhananov R, Crosby G: **Impaired acquisition of spatial memory 2 weeks after isoflurane and isoflurane-**

nitrous oxide anesthesia in aged rats. *Anesth Analg* 2004, **99**:1393-7, table of contents.
62. Bianchi SL, Tran T, Liu C, *et al*: **Brain and behavior changes in 12-month-old Tg2576 and nontransgenic mice exposed to anesthetics.** *Neurobiol Aging* 2008, **29**:1002-10.
63. Eckenhoff RG, Johansson JS, Wei H, *et al*: **Inhaled anesthetic enhancement of amyloid-beta oligomerization and cytotoxicity.** *Anesthesiology* 2004, **101**:703-9.
64. Xie Z, Culley DJ, Dong Y, *et al*: **The common inhalation anesthetic isoflurane induces caspase activation and increases amyloid beta-protein level in vivo.** *Ann Neurol* 2008, **64**:618-27.
65. Xie Z, Dong Y, Maeda U, *et al*: **The common inhalation anesthetic isoflurane induces apoptosis and increases amyloid beta protein levels.** *Anesthesiology* 2006, **104**:988-94.
66. Brambrink AM, Evers AS, Avidan MS, *et al*: **Isoflurane-induced neuroapoptosis in the neonatal rhesus macaque brain.** *Anesthesiology* 2010, **112**:834-41.
67. Planel E, Bretteville A, Liu L, *et al*: **Acceleration and persistence of neurofibrillary pathology in a mouse model of tauopathy following anesthesia.** *FASEB J* 2009, **23**:2595-604.
68. Zhang Y, Dong Y, Wu X, *et al*: **The mitochondrial pathway of anesthetic isoflurane-induced apoptosis.** *J Biol Chem* 2010, **285**:4025-37.
69. Wu X, Lu Y, Dong Y, Zhang G, Zhang Y, Xu Z, Culley D, Crosby G, Marcantonio ER, Tanzi RE, Xie Z: **The inhalation anesthetic isoflurane increases levels of proinflammatory cytokine TNF-alpha, IL-6 and IL-1beta.** *Neurobiol Aging* 2011.
70. Xie Z, Dong Y, Maeda U, *et al*: **The inhalation anesthetic isoflurane induces a vicious cycle of apoptosis and amyloid beta-protein accumulation.** *J Neurosci* 2007, **27**:1247-54.
71. Xie Z, Dong Y, Maeda U, *et al*: **Isoflurane-induced apoptosis: a potential pathogenic link between delirium and dementia.** *J Gerontol A Biol Sci Med Sci* 2006, **61**:1300-6.
72. Xie Z, Dong Y, Maeda U, Xia W, Tanzi RE: **RNA interference silencing of the adaptor molecules ShcC and Fe65 differentially affect amyloid precursor protein processing and Abeta generation.** *J Biol Chem* 2007, **282**:4318-25.
73. Xie Z, Romano DM, Kovacs DM, Tanzi RE: **Effects of RNA interference-mediated silencing of gamma-secretase complex components on cell sensitivity to caspase-3 activation.** *J Biol Chem* 2004, **279**:34130-7.
74. Xie Z, Romano DM, Tanzi RE: **Effects of RNAi-mediated silencing of PEN-2, APH-1a, and nicastrin on wild-type vs FAD mutant forms of presenilin 1.** *J Mol Neurosci* 2005, **25**:67-77.
75. Xie Z, Romano DM, Tanzi RE: **RNA interference-mediated silencing of X11alpha and X11beta attenuates amyloid beta-protein levels via differential effects on beta-amyloid precursor protein processing.** *J Biol Chem* 2005, **280**:15413-21.
76. Zhang G, Dong Y, Zhang B, *et al*: **Isoflurane-induced caspase-3 activation is dependent on cytosolic calcium and can be attenuated by memantine.** *J Neurosci* 2008, **28**:4551-60.
77. McConlogue L, Buttini M, Anderson JP, *et al*: **Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP Transgenic Mice.** *J Biol Chem* 2007, **282**:26326-34.
78. Devi L, Ohno M: **Genetic reductions of beta-site amyloid precursor protein-cleaving enzyme 1 and amyloid-beta ameliorate impairment of conditioned taste aversion memory in 5XFAD Alzheimer's disease model mice.** *Eur J Neurosci* 2010, **31**:110-8.
79. Kimura R, Devi L, Ohno M: **Partial reduction of BACE1 improves synaptic plasticity, recent and remote memories in Alzheimer's disease transgenic mice.** *J Neurochem* 2010, **113**:248-61.
80. Culley DJ, Baxter MG, Yukhananov R, Crosby G: **Long-term impairment of acquisition of a spatial memory task following isoflurane-nitrous oxide anesthesia in rats.** *Anesthesiology* 2004, **100**:309-14.
81. Saab BJ, Maclean AJ, Kanisek M, *et al*: **Short-term memory impairment after isoflurane in mice is prevented by the alpha5 gamma-aminobutyric acid type A receptor inverse agonist L-655,708.** *Anesthesiology* 2010, **113**:1061-71.
82. Wei H, Kang B, Wei W, *et al*: **Isoflurane and sevoflurane affect cell survival and BCL-2/BAX ratio differently.** *Brain Res* 2005, **1037**:139-47.
83. Xu X, Feng J, Zuo Z: **Isoflurane preconditioning reduces the rat NR8383 macrophage injury induced by lipopolysaccharide and interferon gamma.** *Anesthesiology* 2008, **108**:643-50.
84. Li L, Peng L, Zuo Z: **Isoflurane preconditioning increases B-cell lymphoma-2 expression and reduces cytochrome c release from the mitochondria in the ischemic penumbra of rat brain.** *Eur J Pharmacol* 2008, **586(1-3)**:106-13.
85. Raphael J, Zuo Z, Abedat S, Beeri R, Gozal Y: **Isoflurane preconditioning decreases myocardial infarction in rabbits via up-regulation of hypoxia inducible factor 1 that is mediated by mammalian target of rapamycin.** *Anesthesiology* 2008, **108**:415-25.
86. Zaugg M, Jamali NZ, Lucchinetti E, Shafiq SA, Siddiqui MA: **Norepinephrine-induced apoptosis is inhibited in adult rat ventricular myocytes exposed to volatile anesthetics.** *Anesthesiology* 2000, **93**:209-18.
87. Tyther R, Fanning N, Halligan M, Wang J, Redmond HP, Shorten G: **The effect of the anaesthetic agent isoflurane on the rate of neutrophil apoptosis in vitro.** *Ir J Med Sci* 2001, **170**:41-4.
88. Wise-Faberowski L, Raizada MK, Sumners C: **Oxygen and glucose deprivation-induced neuronal apoptosis is attenuated by halothane and isoflurane.** *Anesth Analg* 2001, **93**:1281-7.
89. Wise-Faberowski L, Aono M, Pearlstein RD, Warner DS: **Apoptosis is not enhanced in primary mixed neuronal/glial cultures protected by isoflurane against N-methyl-D-aspartate excitotoxicity.** *Anesth Analg* 2004, **99**:1708-14, table of contents.
90. de Klaver MJ, Manning L, Palmer LA, Rich GF: **Isoflurane pretreatment inhibits cytokine-induced cell death in cultured rat smooth muscle cells and human endothelial cells.** *Anesthesiology* 2002, **97**:24-32.
91. Kawaguchi M, Drummond JC, Cole DJ, Kelly PJ, Spurlock MP, Patel PM: **Effect of isoflurane on neuronal apoptosis in rats subjected to focal cerebral ischemia.** *Anesth Analg* 2004, **98**:798-805, table of contents.
92. Gray JJ, Bickler PE, Fahlman CS, Zhan X, Schuyler JA: **Isoflurane neuroprotection in hypoxic hippocampal slice cultures involves increases in intracellular Ca2+ and mitogen-activated protein kinases.** *Anesthesiology* 2005, **102**:606-15.
93. Lin D, Feng C, Cao M, Zuo Z: **Volatile Anesthetics May Not Induce Significant Toxicity to Human Neuron-Like Cells.** *Anesth Analg* 2010.
94. Pan C, Xu Z, Dong Y, Zhang Y, Zhang J, McAuliffe S, Yue Y, Li T, Xie Z: **The potential dual effects of anesthetic isoflurane on hypoxia-induced caspase-3 activation and increases in BACE levels.** *Anesth Analg* 2011.
95. Xu Z, Dong Y, Wu X, Zhang J, McAuliffe S, Pan C, Zhang Y, Ichinose F, Yue Y, Xie Z: **The potential dual effects of anesthetic isoflurane on Aβ-induced apoptosis.** *Curr Alzheimer Res* 2011.
96. Wei H, Liang G, Yang H: **Isoflurane preconditioning inhibited isoflurane-induced neurotoxicity.** *Neurosci Lett* 2007, **425**:59-62.

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