signal is significantly lost in mature astrocytes in the stratum radiatum of hippocampus as early as 3 h after cerebral ischemic stress in adult mice brain pre-treated with Z-VAD-fmk (Fig. 4e and g), whereas higher reactivity was observed in astrocytes in the PBS-pre–treated (vehicle) ischemic brain (Fig. 4d and g), an indication of ProTα release from astrocytes. Following the combined pre-treatment with Z-VAD-fmk, amlexanox and subsequent ischemia, the signals in astrocytes were recovered as the level in vehicle at 3 h, indicates that Z-VAD-fmk-induced ProTα release from astroglia is blocked by amlexanox (Fig. 4f and g). We also found the similar effects of Z-VAD-fmk and amlexanox on GFAP-positive astroglial ProTα in the ipsilateral the striatum (data are not shown) and somatosensory cortex (Fig. 4j).

No ProTα release from microglia

To see cerebral ischemia-induced ProTα expression in microglia, coronal brain sections were co-stained with anti-ProTα IgG and antibody against Iba-1 (a microglia marker). Recently, we reported that ProTα is localized in both cell body and cytosolic space of processes in microglial in the adult mice brain (Halder and Ueda 2012). Our immunohistochemical analysis suggested that ProTα is also distributed in whole cell in microglia in the adult and neonatal mice brain (Fig. 5a and f). Using the adult brain, the ProTα reactivity was still observed with higher intensity in Iba-1-positive microglia in the ipsilateral stratum radiatum of hippocampus as early as 3 h after ischemia (1 h tMCAO) and reperfusion (Fig. 5b), compared with the control brain (Fig. 5a). However, the ProTα signals were increased gradually in microglia through 24 h (Fig. 5c). On the other hand, there was no effect on microglial ProTα levels by pre-treatment with Z-VAD-fmk in ischemic brain (Fig. 5e), compared with the intensity in the PBS-pre–treated (vehicle) ischemic brain (Fig. 5d). Similar results of ischemia-induced ProTα expression in Iba-1-positive adult microglia were also observed at 3 h and 24 h after ischemia in the regions of striatum (Fig. 5g) and somatosensory cortex (Fig. 5h and i), the regions from where the ProTα signals were completely lost in neurons.

Neuronal depletion of S100A13, a cargo protein for non-classical release

Recently, *in vitro* experiments demonstrated that the non-classical release of ProTα requires interaction with C-terminal sequence of S100A13, a cargo protein (Matsunaga and Ueda 2010). To examine the phenomenon whether S100A13 is released from neurons of adult brain under cerebral ischemic stress (1 h tMCAO), coronal brain sections were co-stained with anti-S100A13 and anti-NeuN antibodies. Our immunostaining data revealed that S100A13 is expressed in NeuN-positive neurons in the CA1 pyramidal cell layer of hippocampus (Fig. 6a), and also in neurons in the striatum (Fig. 6i) and somatosensory cortex (Fig. 6j) of mice brain. As early as 3 h after ischemia and reperfusion, S100A13 was released completely from ipsilateral NeuN-positive CA1 pyramidal neurons (Fig. 6b), compared with the control (Fig. 6a). However, the dot-like signals were observed in some non-neuronal cells in the stratum radiatum of hippocampus (Fig. 6b). S100A13 in pyramidal neurons was recovered in a lesser level at 24 h after ischemia, whereas non-neuronal cells in stratum radiatum completely released S100A13 at this time point (Fig. 6c). Similar results of S100A13 release were observed in ipsilateral neurons of striatum (Fig. 6i) and somatosensory cortex at 3 h (Fig. 6j and k) as well as recovery in neurons at 24 h (Fig 6k) after cerebral ischemic stress.

Blockade of neuronal S100A13 release by amlexanox

It has been described previously that the non-classical release of S100A13 from C6 glioma cells is blocked by amlexanox upon serum-deprivation stress (Matsunaga and Ueda 2010). To investigate the *in vivo* effect of amlexanox on the stress-induced non-classical release of S100A13 from adult brain, mice were treated with amlexanox (10 μg/5 μL; i.c.v.) 30 min before cerebral ischemia (1 h tMCAO) and reperfusion. Using anti-S100A13 and anti-NeuN antibodies, our immunohistochemical findings suggested that S100A13 signals are completely lost in ipsilateral NeuN-positive neurons in the CA1 pyramidal cell layer of hippocampus at 3 h after ischemia in PBS-pre–treated (vehicle) brain (Fig. 6e), retaining the normal staining in the control brain (Fig. 6d). Whereas, S100A13 reactivity was rescued in ipsilateral NeuN-positive CA1 neurons in the ischemic brain pre-treated with amlexanox, an indicative of non-classical blockade of neuronal S100A13 release by amlexanox (Fig. 6f). In confocal microscopy observation, we found that S100A13 immunoreactivity was increased in NeuN-positive CA1 pyramidal neurons of hippocampus due to the blockade of its release by amlexanox (Fig. 6h), compared with the control (Fig. 6g). This result suggests that ischemic stress might cause the up-regulation of S100A13, as shown in Fig. 6c. We also observed the similar results of amlexanox effect on the release of neuronal S100A13 in the striatum (Fig. 6i) and somatosensory cortex (Fig. 6j and k) after the onset of cerebral ischemic stress.

S100A13 is released from astrocytes, but not from microglia

To find out whether S100A13 is released from non-neuronal astrocytes and microglia in the adult brain under cerebral ischemic stress (1 h tMCAO), coronal brain sections were co-stained with anti-S100A13 and antibodies against GFAP and Iba-1. Our double immunostaining data showed that S100A13 is expressed in GFAP-positive astrocytes in the adult mice brain including stratum radiatum of hippocampus (Fig. 7a and c). Following cerebral ischemia and reperfusion, S100A13 signals were partially lost at 3 h in the GFAP-

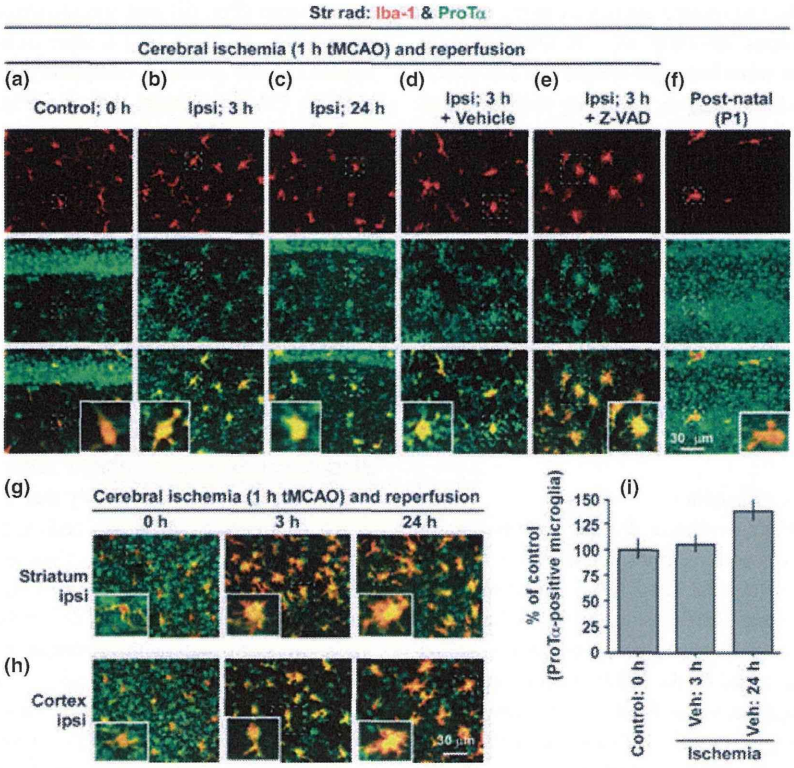

Fig. 5 Microglia have no tendency to release ProTα. (a–f) Coronal brain sections are co-stained with antibodies against ProTα and Iba-1, a microglial marker. (a) Immunohistochemical data shows the expression of ProTα in Iba-1-positive microglia in the stratum radiatum of hippocampus in control brain (Iba-1, red; ProTα, green). (b) ProTα staining is still found with higher intensity in Iba-1-positive microglia in the ipsilateral stratum radiatum at 3 h after ischemic stress. (c) ProTα intensity is increased gradually in Iba-1-positive microglia through 24 h after ischemic stress. (d, e) PBS (5 μL) and Z-VAD-fmk (Z-VAD) at a dose of 1 μg/5 μL (i.c.v.) are injected in the brain 30 min before cerebral ischemia (1 h tMCAO). (d) ProTα reactivity is still found with higher intensity in Iba-1-positive microglia in the ipsilateral stratum radiatum at 3 h after ischemic stress in the PBS-pre–treated (vehicle) brain. (e) Following Z-VAD-fmk pre-treatment, intense ProTα signal is also observed in Iba-1-positive microglia in the stratum radiatum at 3 h after ischemic stress, indicates that ProTα is not released from microglia. (f) ProTα is expressed both in whole Iba-1-positive microglia in the stratum radiatum of hippocampus of post-natal (P1) brain. (g, h) ProTα is not released from micrglia in the striatum and somatosensory cortex after cerebral ischemia. ProTα signal is found with higher intensity in Iba-1-positive micrglia in the ipsilateral striatum and somatosensory cortex at 3 h after ischemic stress, compared with the ProTα signals in the control brain. ProTα intensity is gradually increased in Iba-1-positive micrglia in the ipsilateral striatum and somatosensory cortex through 24 h after ischemic stress. Insets indicate the higher magnification view of ProTα expression in microglia noted by dotted squares. (i) Quantitative analysis of ProTα-positive microglias in the somatosensory cortex. Data represent the means ± SEM.

positive astrocytes of ipsilateral stratum radiatum (data are not shown), followed by completely lost at 24 h after cerebral ischemia (Fig. 7b and d). Similar results of astroglial S100A13 release were observed in the striatum (data are not shown) and somatosensory cortex (Fig. 7e).

However, S100A13 immunoreactivity was absent in Iba-1-positive microglia in the stratum radiatum of normal adult brain (Fig. 7f). We found the similar results of S100A13 absence in the adult microglia of non-ischemic (control) brain using antigen retrieval microwave technique and proteinase K treatment (Fig. 7h and i, respectively). Our findings also suggested that S100A13 is not expressed in Iba-1-positive microglia in the stratum radiatum of post-natal (P1) brain (Fig. 7j). Interestingly, S100A13 signals were absent in Iba-1-positive microglia in the stratum radiatum through 24 h after ischemic stress (Fig. 7g). We found the similar lack of S100A13 expression in Iba-1-positive microglia in the striatum and somatosensory cortex of non-ischemic adult and neonatal brain (data are not shown).

Discussion

ProTα, a signal peptide-deficient nuclear protein, has been identified as a unique cell death regulatory molecule in that it converts the intractable necrosis into the controllable apoptosis (Ueda and Fujita 2004; Ueda *et al.* 2007). This apoptosis is inhibited by brain-derived neurotrophic factor (Ueda 2008). In addition, ProTα potentially inhibits cerebral

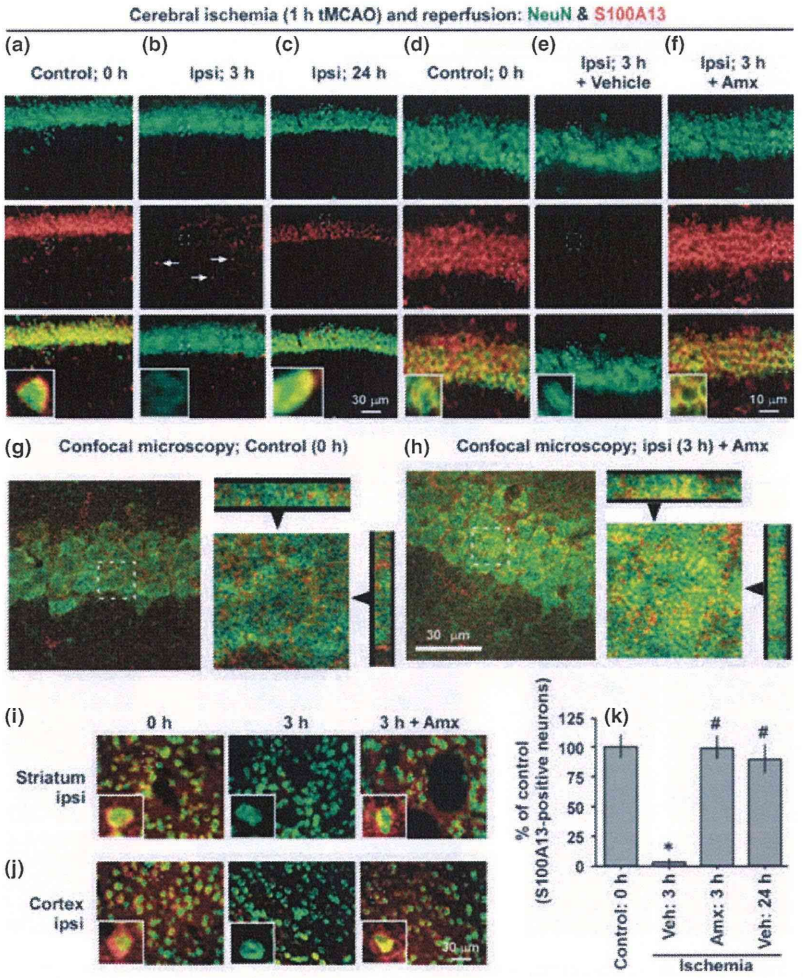

Fig. 6 Amlexanox reversibly blocks the ischemia-induced release of S100A13 from neurons. PBS (5 μL) as well as amlexanox (Amx) at a dose of 10 μg/5 μL (i.c.v.) is injected in the mice brain 30 min before cerebral ischemia (1 h MCAO). (a–j) Double fluorescence immunohistochemical analysis of coronal brain sections is performed using antibodies against S100A13 and NeuN, a neuronal marker. (a) S100A13 is expressed in NeuN-positive CA1 pyramidal neurons of hippocampus in the control brain (NeuN, green; S100A13, red). (b) S100A13 signal is completely lost in NeuN-positive neurons in the ipsilateral CA1 pyramidal cell layer of hippocampus at 3 h after cerebral ischemia. Whereas, the dot-like signals are found in some non-neuronal cells in the stratum radiatum of hippocampus at 3 h indicated by arrows (b). (c) S100A13 is recovered with a lesser intensity in the ipsilateral NeuN-positive CA1 pyramidal neuronal cells at 24 h after ischemic stress, but the signal is completely lost in non-neuronal cells in the stratum radiatum at that time point. (d) S100A13 signal is found in NeuN-positive CA1 pyramidal neurons in the control brain. (e) S100A13 signal is completely lost at 3 h in NeuN-positive CA1 pyramidal neurons in the ipsilateral hippocampus of PBS-pre-treated (vehicle) ischemic brain. (f) Amx pre-treatment blocks S100A13 release from ipsilateral CA1 pyramidal neurons at 3 h after ischemic stress. (g, h) Conforcal microscopy observation in CA1 pyramidal neurons. A higher magnification view is indicated as doted square in panels (g) and (h), respectively. Arrowheads indicate the 3D imaged line (thickness: 10 μm), as shown in upper (*x*-axis) and right panels (*y*-axis). (i, j) Amlexanox inhibits S100A13 release from striatal and somatosensory cortical neurons. S100A13 is expressed in NeuN-positive neurons in the striatum and somatosensory cortex at 0 h as control. S100A13 signal is completely lost in NeuN-positive neurons in the striatum and cortex at 3 h after ischemia. Amx pre-treatment blocks S100A13 release from striatal and cortical neurons at 3 h after ischemic stress respectively. Insets indicate the higher magnification view of S100A13 expression neurons noted by squares. (k) Quantitative analysis of S100A13-positive neuons in the somatosensory cortex. Data represent the means ± SEM ($^{*,\#}p < 0.01$, vs. the control: 0 h and the Amx: 3 h, respectively).

and retinal ischemia-induced necrosis as well as apoptosis (Fujita and Ueda 2007; Fujita *et al.* 2009). Ischemia-specific and ProTα-induced up-regulation of brain-derived neurotrophic factor or erythropoietin is found to contribute to this apoptosis inhibition (Fujita *et al.* 2009; Ueda 2009). Taken together the exclusive findings that the pre-treatments with antisense oligodeoxynucleotide or antibody against ProTα deteriorated the retinal ischemic damages (Fujita *et al.* 2009;

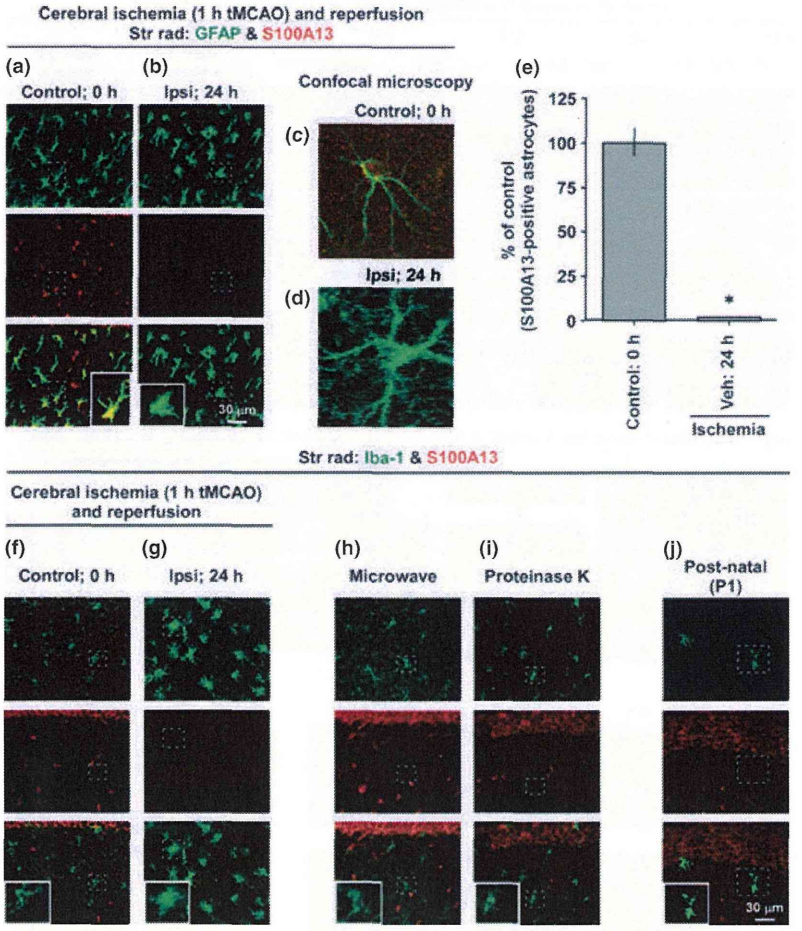

Fig. 7 Ischemia-induced release of S100A13 from astrocytes, but not from microglia. Coronal brain sections are co-stained with antibodies against S100A13, GFAP and Iba-1. (a) Double immunofluorescence staining indicates that S100A13 is expressed in GFAP-positive astrocytes in the stratum radiatum of hippocampus in the control brain (GFAP, green; S100A13, red). (b) Following cerebral ischemia and reperfusion (1 h tMCAO), S100A13 signal is lost completely at 24 h in GFAP-positive astrocytes in the stratum radiatum. (c, d) A higher magnification view of S100A13 in astrocytes. Images were collected by a conforcal microscopy. (e) Quantitative analysis of S100A13-positive astrocytes in the somatosensory cortex. Data represent the means ± SEM. (*$p < 0.01$, vs. the control: 0 h). (f) S100A13 reactivity is absent in Iba-1-positive microglia in the stratum radiatum of normal adult brain (Iba-1, green; S100A13, red). (g) S100A13 is not expressed in Iba-1-positive microglia in the stratum radiatum through 24 h after ischemic stress. (h, i) Antigen retrieval microwave technique (h) as well as proteinase K method (i) followed by double fluorescence immunostaining indicates that S100A13 is not expressed in Iba-1-positive microglia in the stratum radiatum of hippocampus in the adult control brain. (j) Immunostaining data show the lack of S100A13 expression in Iba-1-positive microglia in the stratum radiatum of post-natal (P1) control brain. Insets indicate the high-magnification view of S100A13 expression in astrocyte and microglia noted by dotted squares.

Ueda *et al.* 2010), it is evident that ProTα is a key neuroprotective molecule against ischemic damages.

In the present study, the novel *in vivo* findings include the followings: (i) ProTα is non-classically released along with S100A13 from neuronal cells in the CA1 pyramidal cell layer of hippocampus, striatum and somatosensory cortex of adult brain at 3 h after cerebral ischemia; (ii) amlexanox reversibly blocks this non-classical neuronal ProTα release as well as S100A13; (iii) there is no ProTα release from adult astrocytes and microglia after ischemic stress in brain, followed by gradual up-regulation of ProTα signals in these non-neuronal cells through 24 h; (iv) caspase 3 inhibition by Z-VAD-fmk pre-treatment induces ProTα release from astrocytes as early as 3 h after cerebral ischemia, but this release is reversibly blocked by amlexanox; and (v) ischemia-induced ProTα distribution in microglia is not affected either by Z-VAD-fmk or amlexanox.

The recent *in vitro* study explained that ProTα is localized in the nuclei of cultured cortical neurons and embryonic astrocytes, and that is released from these cells into the extracellular

space upon serum-deprivation stress (Matsunaga and Ueda 2010). This study also suggested that the non-vesicular release of ProTα is initiated through the interaction with S100A13 in the serum-deprived C6 glioma cells *in vitro*. Most recently, we demonstrated that ProTα is strictly localized in nuclei of neuronal cells in adult brain, while it is found in both nuclei and cytosilic space of processes in the astrocytes and microglia (Halder and Ueda 2012). In the present study, we confirmed that ProTα is also localized both in cell body and processes in astrocytes and microglia in the neonatal mice brain, an indication of difference between the brain and culture cell experiments in terms of ProTα expression in astrocytes.

The current study suggested that ProTα is completely released from only neurons in the brain and followed by recovery the signal strictly in the neuronal nuclei after cerebral ischemia, suggesting that the active mechanism may be involved in the epigenetic regulation of ProTα gene expression in neurons of brain under stress condition. It has been reported previously that amlexanox, a potent inhibitor of S100A13, blocks the non-vesicular release of ProTα as well as S100A13 from C6 glioma cells under serum-deprivation stress *in vitro*, due to loss of interaction of S100A13 with C-terminal sequence of ProTα (Matsunaga and Ueda 2010). This *in vivo* study demonstrated that ischemia-induced neuronal ProTα release is reversibly blocked by amlexanox and the signals are diffused in the cytosol of neurons. However, S100A13 was distributed in both cell body and cytosol, and released at the same time point as like ProTα from neurons after the onset of cerebral ischemia. Interestingly, this S100A13 release was in turn blocked in neurons, as the same time point as ProTα released was inhibited, in the amlexanox pre-treated ischemic brain. Together, it can be hypothesized that ProTα is non-classically co-released with S100A13 from neuronal cells in the adult brain under ischemic stress *in vivo*.

At the later time, we characterized the phenomenon whether ProTα is released from non-neuronal cells after cerebral ischemia. We found that none of ProTα is released from astrocytes and microglia after the ischemic stress in brain. Indeed, ProTα intensity was gradually increased in astrocytes and microglia localized in the ischemic regions of brain through 24 h after ischemia. Interestingly, Z-VAD-fmk (caspase 3 inhibitor) pre-treatment induced ProTα release from astrocytes in the ischemic brain, but this release was reversibly blocked by amlexanox. However, S100A13 was expressed in astrocytes of normal brain and released partially at 3 h, followed by complete release at 24 h after brain ischemia. Several *in vitro* studies suggested that the fragmentation of ProTα is mediated by active caspase 3 at the C-terminal sequence located within the spacer region bipartite nuclear localization signal of ProTα (Rubtsov *et al.* 1997; Enkemann *et al.* 2000; Evatafieva *et al.* 2003; Matsunaga and Ueda 2010). The presence of active caspase 3 in the nuclei of astrocytes in adult brain has also been reported *in vivo* (Duran-Vilaregut *et al.* 2010). Recently, it has been demonstrated that S100A13 interacts with C-terminal sequence of ProTα through the C-terminal 11 amino acid peptide sequence of S100A13 in Ca^{2+}-sensitive manner *in vitro*, and that the expression of Δ88–98 mutant of S100A13 selectively inhibits the stress-induced non-classical release of ProTα, but the release of S100A13 mutant itself occurs from C6 glioma cells (Matsunaga and Ueda 2010). This study explains the crucial role of C-terminal peptide sequence of ProTα for non-classical releasing itself. Most recently, we explained that nuclear ProTα level is drastically increased in the astrocytes of non-ischemic brain by Z-VAD-fmk pre-treatment, an indication of active caspase 3-mediated cleavage of C-terminal part possessing nuclear localization signal of ProTα (Halder and Ueda 2012). Therefore, we can explain the possible mechanisms in the following way: (i) astroglial ProTα in the adult brain might loose the capacity to interact with S100A13 due to the cleavage of C-terminal amino acid sequence of ProTα by activated caspase 3 so that no further release is occurred upon brain ischemia; and (ii) the full-length ProTα is redistributed from the cytosol into nuclei of astrocytes in the brain pre-treated with Z-VAD-fmk, followed by consequent release from astrocytes after cerebral ischemic stress *in vivo*. Our findings also suggested that Z-VAD-fmk as well as amlexanox has no effect on the distribution of ProTα in microglia in the ischemic brain. The present study explained the lack of S100A13 expression in microglia of non-ischemic brain, even in brain microglia under the cerebral ischemic stress. Therefore, we can explain one possible mechanism is that microglia loses its capacity to release ProTα from ischemic brain due to the absence of S100A13. However, these findings encourage us to investigate the possible intracellular roles of cytosolic ProTα in astrocytes as well as in microglia. Although there is a close interaction between neurons and non-neuronal cells, astrocytes and microglia are more resistant than neurons to most of ischemic stress (Chen and Swanson 2003; Giffard and Swanson 2005; Trendelenburg and Dirnagl 2005; Rossi *et al.* 2007; Oshiro *et al.* 2008; Lambertsen *et al.* 2009; Faustino *et al.* 2011). There is an interesting report about the ProTα-mediated cellular protection against oxidative stress through the dissociation of the intranuclear Nrf2–Keap1 complex and subsequently facilitation of oxidative stress-protecting genes expression (Karapetian *et al.* 2005). It has also been described that ProTα prevents cells from apoptosis through the inhibition of apoptosome formation (Jiang *et al.* 2003; Letsas and Frangou-Lazaridis 2006). Taken together, our findings indicate the possible *in vivo* role of cytosolic ProTα in astrocytes and microglia in the inhibition of apoptosis.

In the present study, we performed pharmacological inhibitor study against ischemic stress-induced ProTα

release. To confirm our hypothesis of intracellular and extracellular roles of ProTα, we need to perform the study using double knockdown or knockout strategies for S100A13, caspase 3, and caspase 7. As the knockdown strategy using an intracerebroventricular injection is presumed to only partially decrease in the levels of these proteins, the conclusion would not be clear. Although double or triple knockout mice would be perfect to discuss this issue, such mice are not available at present. So, detailed mechanisms would be the next subjects.

Several intracellular proteins lack of conventional signal peptides are released from varieties of cells through non-classical endoplasmic reticulum-Golgi-independent pathways under necrotic/ischemic stress (Gardella *et al.* 2002; Nickel 2005; Prudovsky *et al.* 2008; Nickel and Rabouille 2009; Matsunaga and Ueda 2010). Such a mode for necrotic/ischemic stress-induced extracellular release from neuronal nuclei seems to be similar to the case with HMGB-1, a popular member of DAMPs (Nickel 2005; Faraco *et al.* 2007; Foell *et al.* 2007; Rubartelli and Lotze 2007; Qiu *et al.* 2008). The reciprocal relation from ProTα would be found in the nature that HMGB-1 induces cytotoxic effects *in vitro* and *in vivo* (Scaffidi *et al.* 2002; Lotze and Tracey 2005; Bianchi 2007; Liu *et al.* 2007; Qui *et al.* 2008; Yang *et al.* 2010; Zitvogel *et al.* 2010). However, the pattern of ProTα release from neuronal nuclei is as similar as HMGB-1 release, but dissimilar in the case that ProTα induces robust neuroprotection (Fujita and Ueda 2007; Fujita *et al.* 2009; Ueda *et al.* 2010). Although ProTα-mediated cell survival activity against viral infection through Toll-like receptor-4 has been reported (Mosoian *et al.* 2010), the exact receptor for ProTα signaling is yet unknown. Considering the case, ProTα may be referred as a novel neuroprotective molecule of DAMPs family.

In conclusion, the present study demonstrated that neurons, but not astrocytes and microglias, is the main store of endogenous ProTα, which is released through non-classical pathway upon cerebral ischemia, due to the presence of releasing machineries in neuronal cells in brain. Therefore, the discovery cell type-specific mechanisms of ProTα signaling in the brain may provide a novel solution to protect chronic cellular damages in stroke.

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Supporting information

Additional supporting information may be found in the online version of this article:

Appendix S1. Materials and methods.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

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Issue: *Thymosins in Health and Disease*

Prothymosin α plays multifunctional cell robustness roles in genomic, epigenetic, and nongenomic mechanisms

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Prothymosin α (ProTα) possesses multiple functions for cell robustness. This protein functions intracellularly to stimulate cell proliferation and differentiation through epigenetic or genomic mechanisms. ProTα also regulates the cell defensive mechanisms through an interaction with the Nrf2-Keap1 system. Under the apoptotic conditions, it inhibits apoptosome formation by binding to Apaf-1. Regarding extracellular functions, ProTα is extracellularly released from the nucleus upon necrosis-inducing ischemia stress in a manner of nonclassical release, and thereby inhibits necrosis. However, under the condition of apoptosis, the C-terminus of ProTα is cleaved off and loses binding activity to cargo protein S100A13 for nonclassical release. However, cleaved ProTα is retained in the cytosol and inhibits apoptosome formation. ProTα was recently reported to cause immunological actions through the Toll-like receptor 4. However, the authors also suggest the possible existence of additional receptors for robust cell activities against ischemia stress.

Keywords: linker histone H1; CBP/p300; estrogen receptor; Nrf2-Keap1; Apaf-1; Toll-like receptor-4

Introduction

ProTα is a highly acidic nuclear protein of the α-thymosin family and is found in the nuclei of virtually all mammalian cells.[1,2] ProTα is generally thought to be an oncoprotein that is correlated with cell proliferation by sequestering anticoactivator factor, a repressor of estrogen receptor activity, in various cells.[3,4] With regard to cell death regulation, intracellular ProTα was reported to play a cytoprotective role by inhibiting apoptosome formation in HeLa cells subjected to apoptotic stress.[5] On the other hand, ProTα has been reported to act as an extracellular signaling molecule, as observed in the activation of macrophages, natural killer cells, and lymphokine-activated killer cells, and in the production of IL-2 and TNF-α.[6] ProTα was most recently reported to exert immune responses through Toll-like receptor 4.[7,8] It should be noted that ProTα has potent neuroprotective actions through unique mechanisms by inhibiting neuronal necrosis.[9,10] A recent study revealed the machinery of nonclassical/nonvesicular release of ProTα from the nuclei upon the ischemia/cell starving stress.[11] Thus, there are accumulating findings supporting the conclusion that ProTα plays multiple roles inside and outside of the cell, particularly for cell survival and proliferation.[12]

Nuclear functions

Epigenetic mechanisms

ProTα is highly acidic (pI = 3.55) owing to its abundance of glutamic and aspartic acids (approximately 50% of the total amino acid residues) in the middle part of the protein. The cluster of acidic amino acids in this region seems to resemble a putative histone-binding domain, being consistent with the fact that there is a nuclear localization signal (NLS) at the C-terminal end (human ProTα: KR and KKQK at 87 and 101, respectively). Indeed there are reports that ProTα is highly expressed in many different types of cancer cells,[13–20] and closely related to the cell proliferation and differentiation.[13,21–23] Nuclear ProTα epigenetically stimulates

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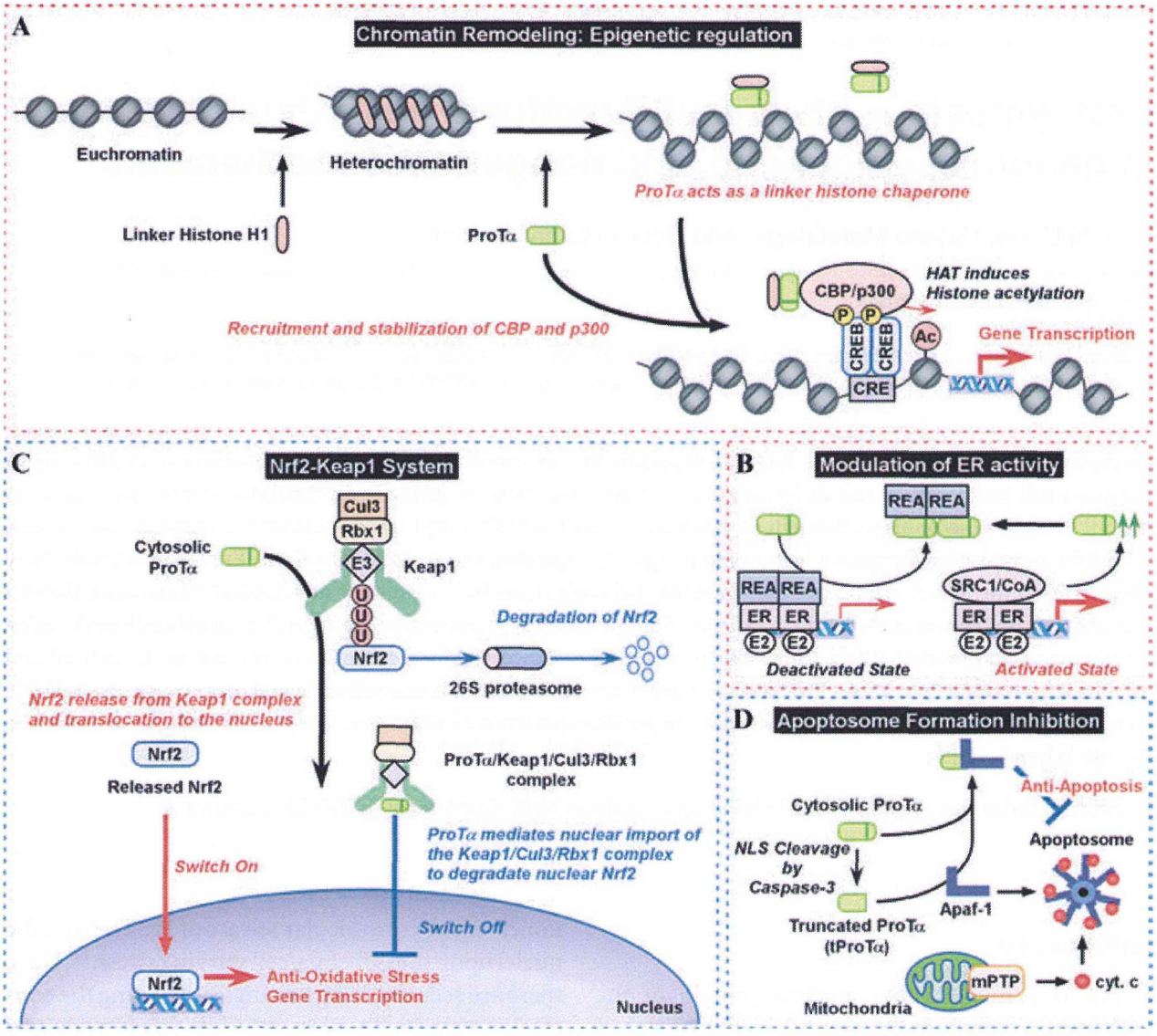

Figure 1. Intracellular multiple functions of ProTα for cell survival and proliferation through a variety of protein–protein interaction. (A) ProTα mediates chromatin remodeling and gene transcription. ProTα acts as a linker histone chaperone. Histone H1 binds to nucleosomal DNA and induces chromatin condensation. ProTα can facilitate H1 displacement from and deposition onto the chromatin template. ProTα is involved in histone acetylation by recruitment and stabilization of p300 histone acetyl transferase (HAT) and CREB-binding protein (CBP). (B) Enhancement of estrogen receptor (ER) transcriptional activity by binding to its repressor (REA: repressor estrogen receptor activity). (C) ProTα regulates the Nrf2-Keap1 system. ProTα binds to Keap1-releasing transcription factor Nrf2, which in turn upregulates antioxidative stress genes involving in apoptosis and autophagy (depicted as switch on). Meanwhile ProTα mediates nuclear import of Keap1/Cul3/Rbx1 complex leads to ubiquitination and degradation of Nrf2 (switch off). (D) ProTα-mediated inhibition of apoptosome by binding of to Apaf-1.

gene transcription by binding to histones,[12] p300 histone acetyltransferase,[24] and CREB-binding protein (CBP).[25] These findings suggests the role of ProTα in the chromatin remodeling (Fig. 1A). According to this hypothesis, ProTα detaches linker histone H1 from chromatin[26] and thus "loosens" it. ProTα also recruits CBP/p300 and stabilizes CBP/p300-CREB complex, resulting in the enhancement of CRE-regulated gene transcription through histone acetylation and chromatin remodeling.

Modulation of estrogen receptor (ER)–mediated transcriptional activity

ProTα enhances the transcriptional activity of estrogen (E2) receptor through a removal of a repressor (estrogen receptor activity (REA)/B cell receptor-associated protein BAP37/prohibitin-2).[3,27] In the