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Primary research on changes of ASPP family in the process of neural differentiation from Rhesus embryonic stem cells

CHEN Rui[1,2], LI Rongrong[2], JI Weizhi[2], ZHANG Huatang[2], LU Xin[2,3]

(1. *School of Life Sciences, University of Science and Technology of China, Hefei* 230027, *China*;
2. *Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming* 650223, *China*;
3. *Ludwig Institute for Cancer Research, Oxford University, Oxford OX*3 7*DQ, UK*)

Abstract: To research whether there is any change of ASPP (Apoptosis-stimulating protein of p53) family, including ASPP1, ASPP2 and iASPP, during the process of early development of the Rhesus neural system, and to research the tendency of the change primarily, the process of early development of Rhesus neural system was simulated through induced neural differentiation from rESC (Rhesus embryonic stem cells) in vitro; meanwhile, the changes of ASPP family in cells were detected by Immunohistochemical Staining and western blotting. The results show that changes of ASPP family in cells have been detected in the process of induced neural differentiation from rESC (Rhesus embryonic stem cells) in vitro, especially amounts and modifications, and some tendencies of these changes can be indicated from these results. The results suggest that there is some certain connection between ASPP family and early development of Rhesus neural system. It might be helpful for diagnosis and treatment of neuronal degenerative diseases and tumours with neuronal origin in the future.

Key words: ASPP1; ASPP2; iASPP; neural progenitors; Rhesus embryonic stem cells
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猕猴胚胎干细胞神经分化过程中 ASPP 家族变化的初步研究

陈 锐[1,2], 李荣荣[2], 季维智[2], 张华堂[2], 卢 欣[2,3]

(1. 中国科学技术大学生命科学学院, 安徽合肥 230027; 2. 中国科学院昆明动物研究所, 云南昆明 650223;
3. 英国牛津大学路德维格肿瘤研究所, 英国牛津 OX3 7DQ)

摘要: 为研究肿瘤细胞凋亡调控因子 ASPP(Apoptosis-stimulating protein of p53)家族蛋白(ASPP1, ASPP2, iASPP)在猕猴神经系统细胞早期发育过程中是否存在变化, 并初步研究其变化趋势, 通过体外诱导猕猴胚胎干细胞定向分化为神经前体细胞模拟猕猴神经系统细胞早期发育过程, 并对此过程中细胞内

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Biography: CHEN Rui, male, born in 1983, master. Research field: cell biology. E-mail: chenrui@mail.ustc.edu.cn
Corresponding author: LU Xin, Prof. E-mail: Xin.Lu@ludwig.ox.ac.uk

ASPP 蛋白量进行检测，检测方法使用细胞免疫荧光和 western blotting. 实验初步检测出，肿瘤调控因子 ASPP 家族蛋白在猕猴神经系统细胞早期发育过程中在蛋白量和蛋白分子量上有变化，并且可以初步了解其变化趋势. 该实验结果表明 ASPP 蛋白家族作为肿瘤细胞凋亡调控因子与猕猴神经系统早期发育过程有着密切的关系，这也许对将来治疗神经系统退行性疾病和肿瘤发生有一定帮助.

关键词：ASPP1；ASPP2；iASPP；神经前体细胞；猕猴胚胎干细胞

0 Introduction

The ASPP (Apoptosis-stimulating protein of p53) proteins comprise an evolutionarily conserved family that consists of three members: ASPP1, ASPP2 which are pro-apoptotic and iASPP which is anti-apoptotic. The c-terminus of all ASPP family members contain the signature sequences Ankyrin repeats, SH3 domain and Proline rich region, hence their name, ASPP again. In the human genome, only three genes contain such signature sequences, which are located on three different chromosomes: chromosome 1 for ASPP2, chromosome 14 for ASPP1 and chromosome 19 for iASPP[1]. The most well studied function of the ASPP family is their ability to regulate apoptosis through regulation of p53, and the p53 family members p63 and p73[2-4]. The most conserved member of the ASPP family is iASPP and it plays an important role in protecting germ cells from DNA damage induced apoptosis in *C. elegans*. This function of iASPP is conserved from *C. elegans* to humans[3]. Only one ASPP member has been identified in invertebrates so far. All three ASPP members have been identified in vertebrates, a pattern that is also seen with p53 family[1]. Recently, p63 has also been reported to play a key role in controlling apoptosis in oocytes[5], female germ cells[6]. In addition, an isoform of p63, delta N p63, is specifically expressed in stem cells and it plays a key role in the maintenance of epithelium stem cells in epidermis and thymus[7]. This is achieved through its ability to inhibit apoptosis, a biological process that is crucial during embryonic development and cell differentiation[8].

Since the ASPP family of proteins are common regulators of the p53 family, we wanted to examine the roles the ASPP family of proteins might play during differentiation, specifically, neural differentiation. This was based on the following observations: first, p63 and p73 are two key inducers of apoptosis in sympathetic neurons[9]; secondly, the ASPP family is also known to bind many other proteins in addition to p53, such as bcl-2, p65relA, YAP, IRS1, PP1 (protein phosphatase 1), APCL, and APP-BP1[10-16]. And both APCL and APP-BP1 are known to be involved in signalling pathways that control neural differentiation. APCL is a member of the tumour suppressor APC family and it was originally identified in the brain[12]. It is also a component of the Wnt signalling pathway, a pathway that plays a pivotal role in neural differentiation[17]. APP-BP1 is a binding protein of the amyloid precursor protein (APP), which plays an important role in regulating neuronal death[13]. Finally, ASPP2 deficient mice suffer from developmental defects in CNS[18]. Collectively, these findings suggested that the ASPP family may play a role in controlling neural differentiation. To test this hypothesis, we analysed the ability of embryonic stem cells to differentiate to a defined cell linage in vitro. The amounts and patterns of the ASPP family of proteins in cells were then examined within this experimental system.

1 Materials and methods

1.1 Culture media

R366.4 Rhesus embryonic stem cells (rESC) (a gift from James Thomson) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Invitrogen Corporation, Carlsbad, CA)

supplemented with 15% fetal bovine serum (FBS) (HyClone, Logan, UT), 2 mmol/L glutamine, 0.1 mmol/L β-mercaptoethanol and 1 × nonessential amino acids[19].

MEFs (Mouse Embryonic Fibroblasts), the feeder cells, were cultured in Dulbecco's modified Eagle's medium, supplemented with 10% Newborn Calf Serum (NCS) (Gibco)[20]. Culture medium for neural progenitor cells (NPs) contains neural differentiation culture media (NDCM) (DMEM/F12 [Gibco], 1 × Insulin, Transferrin and Selenium supplement [ITS] [Gibco], 1.0g/L bovine serum albumin [BSA] [Sigma-Aldrich, St. Louis], 1.0g/L glucose [Sigma-Aldrich], 1.0g/L lactose [Sigma-Aldrich], 0.03g/L proline [Sigma-Aldrich], 5 mmol/L glutamine [Sigma-Aldrich], and 2 mmol/L nicotinamide [Sigma-Aldrich])[21], 10 μg/L bFGF (basic fibroblast growth factor) (Chemicon International, Temecula, CA) and 10 μg/L HGF (hepatocyte growth factor) (Chemicon International).

1.2 Culture of rESCs

R366.4 rESC were cultured on a feeder layer of MEFs which had been treated with 5 mg/L Mitomycin C (Sigma-Aldrich, St. Louis) for 1.5 ~ 2 h prior to rESC culture. MEFs were then washed with room-temperature PBS 5 x to remove the Mitomycin C, before rESCs were seeded onto the MEF feeder cells in rESC medium. rESCs colonies were grown for 4~5 d to allow growth to the maximal size without visible differentiation, after which cells were digested by 10 g/L dispase (Gibco). After gently dispersing into smaller colonies, rESCs were seeded onto newly prepared feeder layers of MEFs[20].

1.3 Culture of NPs from rESCs

Prior to differentiation, rESCs colonies were cultured on feeder cells to maximal size at 37 ℃ and then digested with 1 g/L dispase, washed to remove dispase and then suspended in DMEM/F12 media. Colonies that contained nearly 500 ~ 800 cells were transferred to new four-well plates coated with 20 mg/L laminin (Sigma-Aldrich) and seeded to a density of 25~30 cells per cm^2. After 12 h and colony attachment, the medium was replaced with a fresh NPs culture medium. During the proliferation and differentiation of cells, half of the medium was changed every day. After 4~5 d in culture, the proliferating cells covered most of the bottom of plates and were then separated manually by selecting the compact sections or rosette-type sections with a glass needle under the microscope. After washing with NPs culture medium to remove dead cells, the massed sections were seeded onto new plates coated with laminin for continued culturing at 37 ℃.

1.4 Immunohistochemical staining and antibodies

Prior to staining, cells were attached on small circular coverslips in 4-well plates. Coverslips were then submerged in 75% alcohol for 0.5 h, followed by washing 3 times with PBS in the wells of new plates, before coating them with 20 mg/L laminin for 2 h. The wells for rESCs were seeded with MEFs before passaging rESCs in them, to prevent the onset of random differentiation.

The cells on the coverslips were washed with DMEM and fixed with 4% paraformaldehyde (Sangon, Shanghai, CN) in PBS for 20 min at room temperature. After washing with PBS, the cells were penetrated with 0.5% Triton-100 (Sigma-Aldrich) for 20 min and washed with PBS again. Blocking was carried out in a blocking buffer (2% BSA in PBS) for 1 h at room temperature. Primary antibodies were applied in the blocking buffer for 1 h at room temperature or overnight at 4 ℃ and washed three times with PBS before application of the appropriate secondary antibody. Secondary antibodies were diluted in PBS and applied to cells for 1 h at room temperature. After three washes in PBS, hoechest 33 342 was applied for 20 min for nuclear counterstaining and cells observed under the fluorescence microscope. For negative controls, primary antibodies were omitted and the same staining procedure was followed.

For double staining, cells were incubated with

two primary antibodies from different species and visualised with FITC and TRIC labelled secondary antibodies accordingly.

Mouse monoclonal antibodies against ASPP1, ASPP2 and iASPP were LX54.2, LX089 and LX49.3, respectively, and their specificities were characterised as described previously[2,22-23]. The other primary antibodies for immunohistochemical staining were anti-nestin rabbit polyclonal antibody and mouse monoclonal antibody, (Chemicon International), anti-β Ⅲ-tubulin rabbit polyclonal antibody (Abcam plc, Cambridge, UK) and anti-Oct4 rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies for immunohistochemical staining were goat anti-rabbit IgG-FITC (SouthernBiotech, Birmingham), goat anti-rabbit IgG-Texas Red (Santa Cruz Biotechnology) and goat anti-mouse IgG-FITC (Biolegend, San Diego, CA).

1.5 Western blotting and antibodies

Cells were lysed in Urea buffer (8 mol/L Urea [BBI, Ontario, CA], 1 mol/L Thiourea [BBI], 0.5% CHAPS [BBI], 50 mmol/L DTT [BBI] and 24 mmol/L Spermine [Sigma-Aldrich]). The protein concentration of the extract was detected by Bradford protein assay. Between 15 and 30 μg of extract was mixed with 5 × sample buffer, boiled for 10 min and loaded onto 8% SDS-PAGE gels. Protein was then transferred from the gel onto PVDF membrane. The resulting membranes were blocked with 5% non-fat milk or 5% BSA and incubated with primary antibodies, before incubating with the appropriate secondary HRP-conjugated antibody. After washing, the membrane was exposed to medical X-ray films (Lucky Corporation, CN) immediately following the use of SuperSignal West Pico Chemiluminescent Substrate (Pierce).

LX54.2, LX089 and LX49.3 mouse monoclonal antibodies were used to detect ASPP1, ASPP2 and iASPP respectively. Mouse monoclonal anti-βactin antibody (Abcam) was used to detect βactin. Horseradish peroxidise conjugated goat anti-mouse IgG was used as a secondary antibody (Pierce Biotechnology, Rockford).

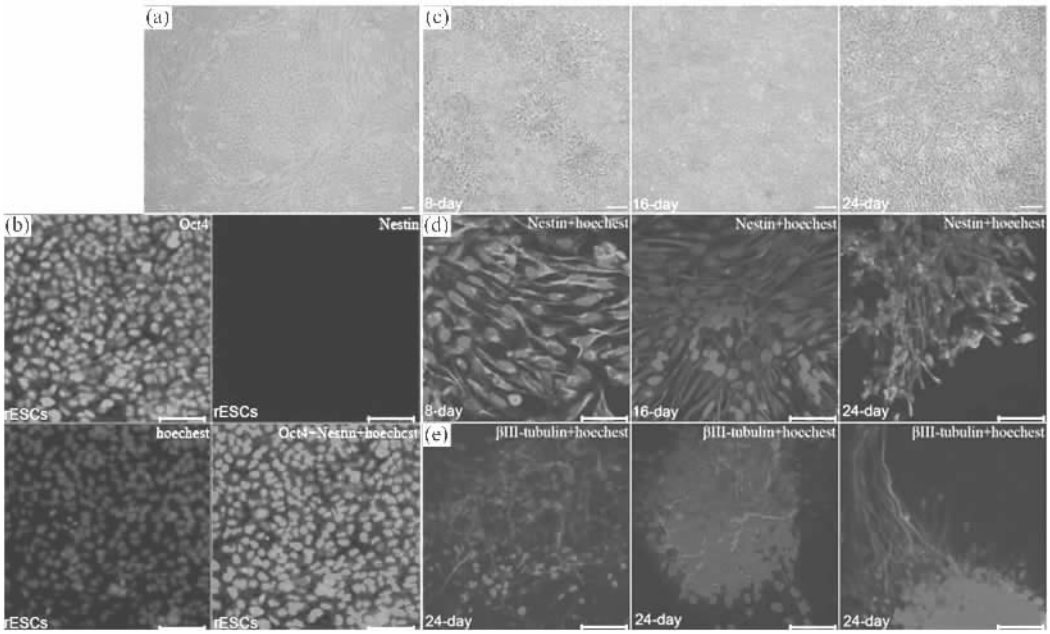
2 Results

2.1 Characterisation of Rhesus embryonic stem cell (rESC) differentiation to neural linage

Rhesus embryonic stem cells (rESC) were cultured on a feeder layer under the conditions as described previously[19]. The pluripotency of the cultured rESC is confirmed by their morphological appearance (Fig.1(a)) and the homogeneous expression of an embryonic stem cell marker, Oct4 (Fig.1(b)). Under the same conditions, they do not express Nestin, a neural intermediate filament protein that labels neuronal epithelial cells or neural progenitors (Fig.1(b)) demonstrating that they are not yet differentiated into neural linage.

The Rhesus embryonic stem cells were isolated and kept in defined adherent culture conditions as described[24-26], which are known to induce neural differentiation. HGF and bFGF were also added to adherent cultures to induce the rESC to differentiate in serum free medium, as previously described. Phase contrast light microscopy examination revealed that the majority of Rhesus embryonic stem cells exhibited rosette-neural structure after 8 d differentiation (Fig.1(c)).

Induced cells were then fixed at 8, 16 and 24 days after the addition of HGF and bFGF. The fixed cells were stained for the expression of proteins which mark different stages of neural differentiation. Consistent with the result seen in Fig.1(c), Nestin positive cells were detected in the majority of Rhesus embryonic stem cells grown in HGF and bFGF containing adherent culture condition for 8 days, indicating that the cultured Rhesus embryonic stem cells have already differentiated into neural progenitors (Fig.1(d)). Nearly all Rhesus embryonic stem cells remained positive for Nestin 16 days after culturing in media containing HGF and bFGF (Fig.1(d)). By 24



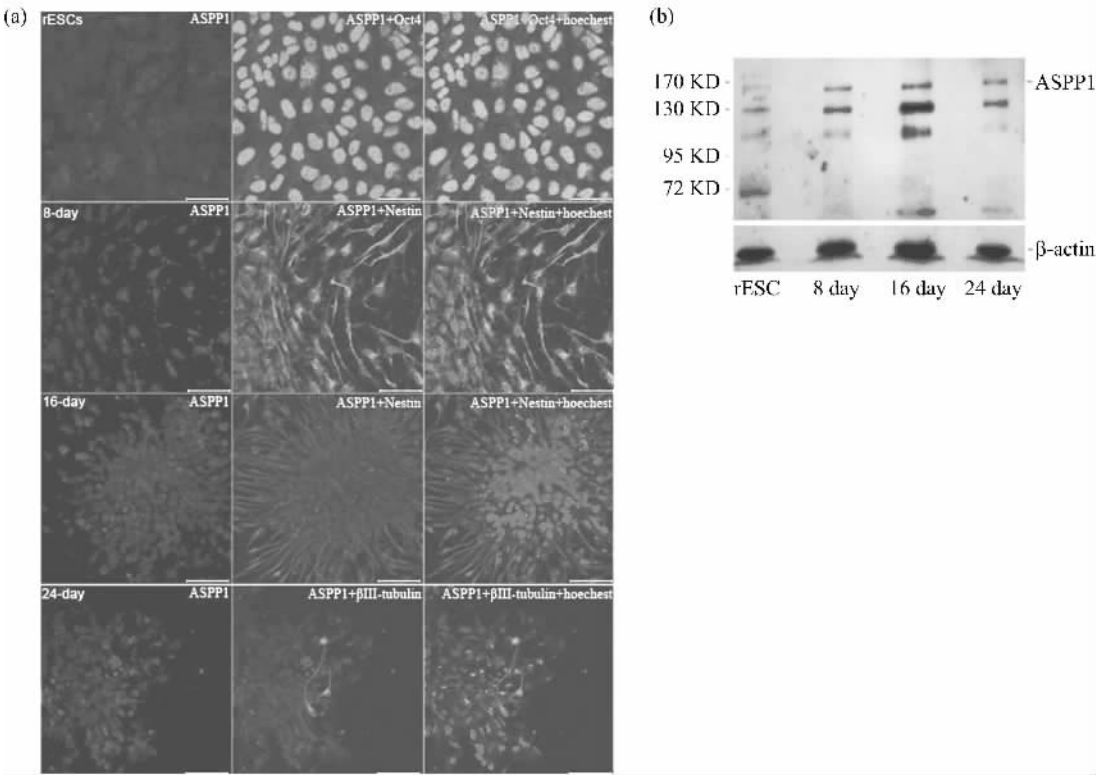
(a) Phase contrast image of rESCs (cell colonies) grown on MEFs which are fibroblast-like cells around the cell colonies at 100× magnification. Cells were imaged following 4 d growth after passaging. White bar represents 50 μm. (b) rESCs were fixed and immunostained for Oct4 and Nestin as described. Oct4, as an embryonic cell marker; Nestin, as a neural progenitors marker. Nuclei were labelled by hoechest 33 342. Cells were imaged at 200× magnification and white bars represent 100 μm. (c) Phase contrast image of rESCs following incubation in media containing HGF and bFGF for 8, 16 and 24 days. Typical rosette-neural progenitors appear on the eighth day with HGF and bFGF treatment. Cells were imaged at 200× magnification and white bars represent 50 μm. (d) Immunostaining of rESCs for Nestin expression following growth in media containing HGF and bFGF for 8, 16 and 24 days. Neural progenitors appear and remain with HGF and bFGF treatment. Cells were counter-stained with hoechest 33342, labelling nuclei. Cells were imaged at 400× magnification and white bars represent 50 μm. (e) Immunostaining of rESCs after 24 days growth in HGF and bFGF containing media, for βⅢ-tubulin and counter-stained with hoechest 33 342. Some parts of neural progenitors begin to differentiate into postmitotic neurons. βⅢ-tubulin, as a postmitotic neuronal marker. Cells were imaged at 400× magnification and white bars represent 50 μm. Abbreviation: rESC, Rhesus embryonic stem cells; MEF, mouse embryonic fibroblast; HGF, hepatocyte growth factor; bFGF, basic fibroblast growth factor.

Fig. 1 Characterisation of Rhesus embryonic stem cell (rESC) differentiation to neural linage

days, however, a small number of cells started to express the post mitotic neuronal marker b-Ⅲ tubulin, indicating that some of the Nestin positive neural progenitors have started to differentiate into postmitotic neurons (Fig. 1(e)). Thus, these time points allowed us to address the question of whether the amounts and patterns of the ASPP family of proteins are changed during the process of neural differentiation.

2.2 Changes of ASPP1 and ASPP2 in the process of neural differentiation from rESC

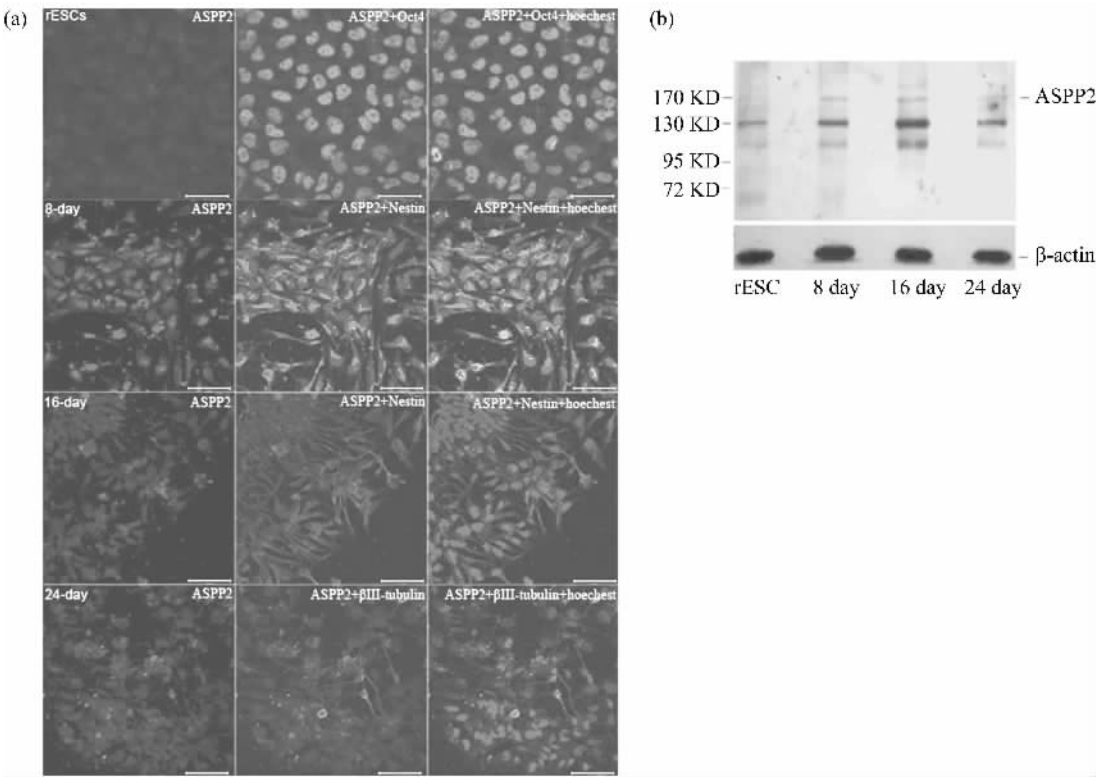
The patterns and amounts of pro-apoptotic ASPP family members, ASPP1 and ASPP2 were first examined in Rhesus embryonic stem cells grown in HGF and bFGF containing adherent cultures conditions for 0, 8, 16 and 24 days. When Rhesus embryonic stem cells were in their pluripotent state, as supported by Oct4 expression, very little ASPP1 and ASPP2 protein expression was detected by both immunofluorescent staining (Fig. 2(a) and Fig. 3(a)) as well as immunoblotting (Fig. 2(b) and Fig. 3(b)). Interestingly, the ASPP1 and ASPP2 amounts increased when Rhesus embryonic stem cells started to express Nestin, a neuronal epithelial cell or neural progenitor marker (Fig. 2(a) and Fig. 3(a)). Nuclear ASPP1 and ASPP2 remained detectable in rESCs for 24 days after



(a) rESCs and rESCs grown in media containing HGF and bFGF for the indicated times, were immunostained for ASPP1 and Oct4, Nestin or βⅢ-tubulin using antibodies as described in the material and methods. Cells were counter-stained using hoechest 33 342, labelling nuclei. Oct4, as an embryonic cell marker; Nestin, as an neural progenitors marker; βⅢ-tubulin, as a postmitotic neuronal marker. Cells were imaged at 400× magnification and white bars represent 50 μm. (b) Immunoblot analysis of ASPP1 expression levels from extracts obtained from rESCs and rESCs grown in media containing HGF and bFGF for the indicated times. Equal loading is demonstrated by β-actin expression levels. Abbreviation: rESC, Rhesus embryonic stem cells; ASPP1, apoptosis-stimulating protein of p53 1; HGF, hepatocyte growth factor; bFGF, basic fibroblast growth factor

Fig. 2　Change of ASPP1 in neural differentiation

induction of neural differentiation. This increased amounts of ASPP1 and ASPP2 in neural differentiation was further examined by a more quantitative immunoblotting assay. Mouse monoclonal anti-ASPP1 and anti-ASPP2 antibodies detected four fragments in rESC cells, which approximately migrate as 170 KD, 130 KD, 100 KD and 70 KD fragments (Fig. 2(b) and Fig. 3(b)). The specificities of the anti-ASPP1 and ASPP2 antibodies were published previously and they were also confirmed using ASPP1 and ASPP2 deficient cells[2]. Based on the mobility of human and mouse ASPP1 and ASPP2 proteins, the full length monkey ASPP1 and ASPP2 should migrate as a 170 KD protein. The nature of the smaller fragments of ASPP1 and ASPP2 detected in this study remains unclear. Interestingly, however, the 70 KD fragment of ASPP1 and ASPP2 was only detected in rESCs and the 130 KD fragment of ASPP1 and ASPP2 was the most prominent fragment detected in rESCs. In the neural differentiation, the disappearance of the 70 KD ASPP1 and ASPP2 was accompanied by an increase in the levels of all three remaining fragments, that is, 170 KD, 130 KD and 100 KD. This agrees with the increase in the number of nuclear ASPP1 and ASPP2 expressing cells detected by immunoflouresence staining (Fig. 2(a) and Fig. 3(a)). A further increase in ASPP1 and ASPP2 amounts was seen in Rhesus embryonic stem cells cultured in HGF and bFGF containing adherent conditions for 16 days, a time point where almost



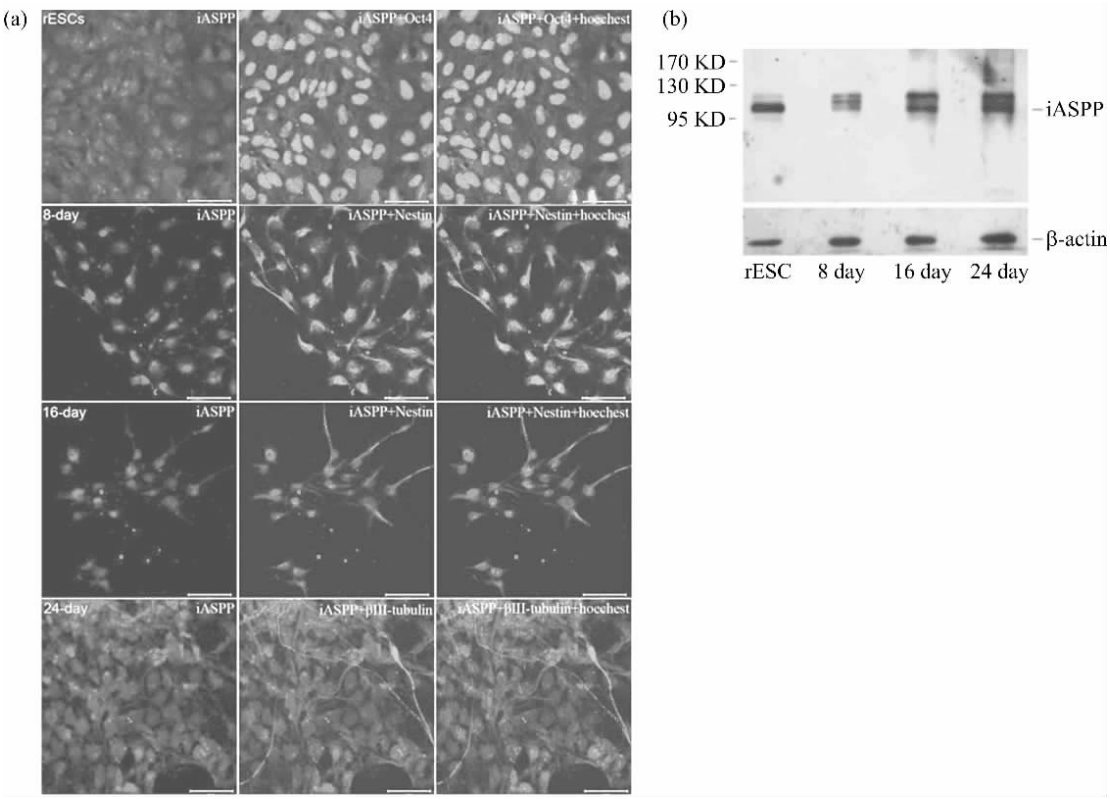
(a) rESCs and rESCs grown in media containing HGF and bFGF for the indicated times, were immunostained for ASPP2 and Oct4, Nestin or βⅢ-tubulin using antibodies as described in Material and Methods. Cells were counter-stained using hoechest 33342, labelling nuclei. Oct4, as an embryonic cell marker; Nestin, as a neural progenitors marker; βⅢ-tubulin, as a postmitotic neuronal marker. Cells were imaged at 400× magnification and white bars represent 50 μm. (b) Immunoblot analysis of ASPP2 expression levels from extracts obtained from rESCs and rESCs grown in media containing HGF and bFGF for the indicated times. Equal loading is demonstrated by β-actin expression levels. Abbreviation: rESC, Rhesus embryonic stem cells; ASPP2, apoptosis-stimulating protein of p53 2; HGF, hepatocyte growth factor; bFGF, basic fibroblast growth factor

Fig. 3 Change of ASPP2 in neural differentiation

all cells expressed Nestin (Fig. 1(d)). A small but consistent reduction of ASPP1 and ASPP2 amounts were detected in Rhesus embryonic stem cells cultured in HGF and bFGF containing medium for 24 days, a time point when some of the cultured Rhesus embryonic stem cells started to express b-Ⅲ tubulin (Fig. 1(e)). These data demonstrate for the first time that the amount levels of the pro-apoptotic ASPP family members, ASPP1 and ASPP2 increase in the neural differentiation.

2.3 Changes of post-translational modification of iASPP in neural differentiation

Unlike ASPP1 and ASPP2, nuclear iASPP was clearly detected in pluripotent Rhesus embryonic stem cells, as demonstrated by positive Oct4 staining. No significant changes in iASPP amount or cellular distribution were detected in Rhesus embryonic stem cells during neural differentiation (Fig. 4 (a)). Interestingly, however, a dramatic change in iASPP mobility was detected by immunoblotting assay. In Rhesus embryonic stem cells, iASPP was predominantly expressed as a 100 KD fragment (Fig. 4(b)). When Rhesus embryonic stem cells start to differentiate into neuronal epithelial cells, as demonstrated by Nestin expression (8 days in HGF and bFGF cultures), two slower migrating fragments of iASPP appear, indicative of post-translational modifications of iASPP. Furthermore, a significant reduction in the level of the 100 KD iASPP fragment was also detected



(a) rESCs and rESCs grown in media containing HGF and bFGF for the indicated times, were immunostained for iASPP and Oct4, Nestin or βⅢ-tubulin using antibodies as described in the material and methods. Cells were counter-stained using hoechest 33342, labelling nuclei. Oct4, as an embryonic cells marker; Nestin, as a neural progenitors marker; βⅢ-tubulin, as a postmitotic neuronal marker. Cells were imaged at 400× magnification and white bars represent 50 μm. (b) Immunoblot analysis of iASPP expression levels from extracts obtained from rESCs and rESCs grown in media containing HGF and bFGF for the indicated times. Equal loading is demonstrated by β-actin expression levels. Abbreviation: rESC, Rhesus embryonic stem cells; iASPP, inhibitory apoptosis-stimulating protein of p53; HGF, hepatocyte growth factor; bFGF, basic fibroblast growth factor

Fig. 4　iASPP is post-translationally modified in neural differentiation

after 8 days of culturing in HGF and bFGF containing media (Fig. 4(b)). The intensity of the slowest migrating fragment of iASPP increased further after 16 days incubation, which was retained in cells up to 24 days (Fig. 4(b)). These results suggest that iASPP activity is likely to be changed by post-translational modifications and that these modifications of iASPP change in neural differentiation.

3　Discussion

Using a defined in vitro neural differentiation system from Rhesus monkey embryonic stem cells, we reported for the very first time that the expression of the evolutionarily conserved ASPP family of proteins is tightly changed during neural differentiation. Existing studies showed that the ASPP family members have opposing functions and they act as Yin and Yang regulators of the tumour suppressor p53. ASPP1 and ASPP2 have similar functions and they are activators of p53 and p53 family members whereas iASPP is an inhibitor of p53 (reviewed in Ref. [1]). Importantly, iASPP is one of the most conserved inhibitors of p53 identified so far. It was therefore very interesting to note that although the amount of all three ASPP members changed in neural differentiation, the changing pattern for the pro-apoptotic ASPP1 and ASPP2 is completely different from that of iASPP.

It was assuring to observe that the change of ASPP1 and ASPP2 is very similar and predominantly at the amount levels of the proteins. Both ASPP1 and ASPP2 were detected at low levels in Rhesus embryonic stem cells but their amount levels increased in neural differentiation (Fig. 2 and Fig. 3). Interestingly, changes in patterns of both ASPP1 and ASPP2 are similar, supporting their functional similarities. And it is likely that there are some functions of ASPP1 and ASPP2 in neural differentiation from rESC, or neural differentiation would affect ASPP1 and ASPP2 expression during this process. All these need further study.

In contrast, the change of iASPP is not at the total protein level. In agreement with the notion that iASPP is a potent inhibitor of apoptosis in C. elegans germ cells, iASPP expression is clearly detectable in Rhesus embryonic stem cells. Instead, post-translational modifications of iASPP are predominantly changed in neural differentiation (Fig. 4). Future studies are needed to determine the precise modifications of iASPP. The biological functions of these modified iASPP isoforms are also of great importance in our future understanding of iASPP biology.

Together, the studies suggest that the ASPP family of proteins may play a critical role in neural cell growth through their abilities to influence neural differentiation and/or apoptosis. Future identification of the fast migrating ASPP1 and ASPP2 isoforms and the slower migrating isoforms of iASPP, which are differentially expressed in embryonic stem cells and different neural cell linages, may provide us with novel biomarkers for stem cell and neural differentiated cells. Understanding the biological importance of these isoforms will extend our knowledge of the physiological functions of the ASPP family of proteins in the development of neuronal degenerative disease and neuronal tumours. The ASPP family of proteins could serve as new biomarkers and novel molecular targets in our future diagnosis and treatment of neuronal degenerative diseases and tumours with neuronal origin.

References

[1] Trigiante G, Lu X. ASPP and cancer[J]. Nature Reviews Cancer, 2006, 6:217-226.

[2] Samuels-Lev Y, O'Connor D J, Bergamaschi D, et al. ASPP proteins specifically stimulate the apoptotic function of p53[J]. Molecular Cell, 2001, 8 (4): 781-794.

[3] Bergamaschi D, Samuels Y, O'Neil N J, et al. iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human[J]. Nature Genetics, 2003, 33: 162-167.

[4] Bergamaschi D, Samuels Y, Jin B, et al. ASPP1 and ASPP2: Common activators of p53 family members [J]. Molecular and Cellular Biology, 2004, 24 (3): 1 341-1 350.

[5] Livera G, Petre-Lazar B, Guerquin M J, et al. p63 null mutation protects mouse oocytes from radio-induced apoptosis[J]. Reproduction, 2008, 135: 3-12.

[6] Suh E K, Yang A, Kettenbach A, et al. p63 protects the female germ line during meiotic arrest[J]. Nature, 2006, 444:624-628.

[7] Senoo M, Pinto F, Crum C P, et al. p63 is essential for the proliferative potential of stem cells in stratified epithelia[J]. Cell, 2007, 129 (3):523-536.

[8] Vousden K H, Lu X. Live or let die: The cell's response to p53[J]. Nature Reviews Cancer, 2002, 2: 594-604.

[9] Jacobs W B, Kaplan D R, Miller F D. The p53 family in nervous system development and disease[J]. Journal of Neurochemistry, 2006, 97 (6):1 571-1 584.

[10] Helps N R, Barker H M, Elledge S J, et al. Protein phosphatase 1 interacts with p53BP2, a protein which binds to the tumour suppressor p53[J]. FEBS Letters, 1995, 377 (3):295-300.

[11] Yang J P, Hori M, Sanda T, et al. Identification of a novel inhibitor of nuclear factor-κB, RelA-associated inhibitor[J]. Journal of Biological Chemistry, 1999, 274 (22):15 662-15 670.

[12] Nakagawa H, Koyama K, Murata Y, et al. APCL, a central nervous system-specific homologue of adenomatous polyposis coli tumor suppressor, binds to p53-binding protein 2 and translocates it to the perinucleus[J]. Cancer Research, 2000, 60:101-105.

[13] Chen Y, Liu W, Naumovski L, et al. ASPP2 inhibits

APP-BP1-mediated NEDD8 conjugation to cullin-1 and decreases APP-BP1-induced cell proliferation and neuronal apoptosis[J]. Journal of Neurochemistry, 2003, 85 (3):801-809.

[14] Espanel X, Sudol M. Yes-associated protein and p53-binding protein-2 interact through their WW and SH3 domains[J]. Journal of Biological Chemistry, 2001, 276 (17):14 514-14 523.

[15] Naumovski L, Cleary M L. The p53-binding protein 53BP2 also interacts with Bc12 and impedes cell cycle progression at G2/M[J]. Molecular and Cellular Biology, 1996, 16 (7):3 884-3 892.

[16] Hakuno F, Kurihara S, Watson R T, et al. 53BP2S, interacting with insulin receptor substrates, modulates insulin signaling[J]. Journal of Biological Chemistry, 2007, 282 (52):37 747-37 758.

[17] Clevers H. Wnt/β-catenin signaling in development and disease[J]. Cell, 2006, 127 (3):469-480.

[18] Vives V, Su J, Zhong S, et al. ASPP2 is a haploinsufficient tumor suppressor that cooperates with p53 to suppress tumor growth[J]. Genes & Development, 2006, 20:1 262-1 267.

[19] Thomson J A, Kalishman J, Golos T G, et al. Isolation of a primate embryonic stem cell line[J]. Proceedings of the National Academy of Sciences of the United States of America, 1995, 92 (17):7 844-7 848.

[20] Li T, Wang Shufen, Xie Yunhua, et al. Homologous feeder cells support undifferentiated growth and pluripotency in monkey embryonic stem cells[J]. Stem Cells, 2005, 23 (8):1 192-1 199.

[21] Li Tianqing, Zheng Jiawei, Xie Yunhua, et al. Transplantable neural progenitor populations derived from rhesus monkey embryonic stem cells[J]. Stem Cells, 2005, 23 (9):1 295-1 303.

[22] Slee E A, Gillotin S, Bergamaschi D, et al. The N-terminus of a novel isoform of human iASPP is required for its cytoplasmic localization[J]. Oncogene, 2004, 23:9 007-9 016.

[23] Fogal V, Kartasheva N N, Trigiante G, et al. ASPP1 and ASPP2 are new transcriptional targets of E2F[J]. Cell Death and Differentiation, 2005, 12:369-376.

[24] Shin S, Mitalipova M, Noggle S, et al. Long-term proliferation of human embryonic stem cell-derived neuroepithelial cells using defined adherent culture conditions[J]. Stem Cells, 2006, 24 (1):125-138.

[25] Ying Q L, Stavridis M, Griffiths D, et al. Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture[J]. Nature Biotechnology, 2003, 21:183-186.

[26] Gerrard L, Rodgers L, Cui W. Differentiation of human embryonic stem cells to neural lineages in adherent culture by blocking bone morphogenetic protein signaling[J]. Stem Cells, 2005, 23 (9):1 234-1 241.