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Neuroscience Letters 374 (2005) 47–52
Neuroscience Letters
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Possible involvement of the expression and phosphorylation of N-Myc in the induction of HMGA1a by hypoxia in the human neuroblastoma cell line

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Received 12 August 2004; received in revised form 8 October 2004; accepted 9 October 2004

Abstract

Increased expression of N-Myc and expression of the high mobility group protein A1a (HMGA1a) were observed in the nuclei of SK-N-SH cells following exposure to hypoxia. These observations were accompanied by the appearance of additional high molecular weight bands, which were eliminated by pretreatment with alkaline phosphatase. Immunoprecipitation showed phosphorylation of serine, threonine and tyrosine residues of N-Myc in the nucleus. These results suggest that hypoxia-induced signals in SK-N-SH cells lead to persistent expression of HMGA1a, which may induce expression of the transcription factor N-Myc, and that phosphorylation at serine, threonine and tyrosine residues of N-Myc occurs at an early stage after stimulation. Such signal consolidation processes could play a role in neuronal survival after hypoxia in neurons.

Keywords: Hypoxia; HMGA1a; Myc family members; Phosphorylation; Neuroblastoma

Alzheimer's disease (AD) is a neurodegenerative disorder that has several pathological characteristics: severe neuron loss, glial proliferation, extracellular deposition of senile plaques composed of beta-amyloid, and deposition of intracellular neurofibrillary tangles [39]. Recently, we discovered that an alternative splice variant of the *presenilin*-2 (*PS*2) gene that lacks exon 5 (PS2V) is significantly expressed in the brains of sporadic AD patients, compared with those of controls [35]. PS2V encodes aberrant proteins that form intracellular inclusion bodies (PS2V bodies) [20], and these are present in pyramidal cells of the cerebral cortex and the hippocampus in 100% of sporadic AD brains [36]. Furthermore, cell lines that express PS2V become fragile in response to various endoplasmic reticulum (ER) stresses [35,36], leading to changes in the conformation of tau proteins [25].

The expression of PS2V observed in sporadic AD is mimicked in hypoxia-exposed human neuroblastoma SK-N-SH cells [35,36]. PS2V is induced by the action of the high mobility group protein A1a (HMGA1a), which directly binds to specific sequences on PS2 pre-mRNA in SK-N-SH cells following hypoxic stimulation [19]. Furthermore, increased expression of the HMGA1a protein has been observed in the hippocampus of sporadic AD brains and nuclear extracts from hypoxia-exposed SK-N-SH cells, compared with those from controls cells under normoxia [19]. The protein has been observed to accumulate in nuclear speckles, along with the endogenous splicing factor SC35 [19].

Abbreviations: HMGA1a, high molecular group protein A1a; PS, phosphoserine; PT, phosphothreonine; PY, phosphotyrosine

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doi:10.1016/j.neulet.2004.10.039

Members of the HMGA protein family participate in many cellular processes, including regulation of inducible gene transcription, integration of retroviruses into chromosomes, and metastatic progression of cancer cells [31]. HMGA proteins contain three copies of a conserved DNA-binding peptide motif called the 'AT-hook', which preferentially binds to the AT-rich DNA minor groove and interacts in vivo with a large number of other proteins, many of which are transcription factors.

A previous study has shown that HMGA1a is a direct c-Myc-targeted gene involved in neoplastic transformation in Burkitt's lymphoma [43]. The Myc family proteins, N-Myc and c-Myc, are implicated in the regulation of cell proliferation, differentiation and apoptosis [5,13,28,29]. It is likely that c-Myc expression is differentially activated in various paradigms of neuronal cell death in vivo and in vitro [1,23,24], and it has been suggested that N-Myc function depends on cell type and developmental stage, as well as on the external environment [3,37,41]. On the other hand, previous reports have demonstrated that N-Myc, c-Myc and phosphorylated c-Myc are present in the AD brain and in brains affected by other neurodegenerative diseases [7,8]. Furthermore, more potent expression of c-Myc protein and c-*myc* mRNA have been observed in ischemic rodent brains [23,24], and previous reports have demonstrated that hypoxia promotes apoptosis of human neuroblastoma cell lines through enhanced N-Myc expression [32].

It is known that the functions and DNA-binding activities of Myc family proteins are regulated by phosphorylation [9–12,14–17,26,27,33,34,40,42]. Furthermore, Myc function requires heterodimerization of the Myc and Max basic/helix–loop–helix/leucine zipper (bHLHZ) domains prior to sequence-specific DNA binding. Myc–Max heterodimers recognize a core hexanucleotide element (5′-CACGTG-3′), termed the Enhancer box or E box [4,30], and activate transcription at promoters containing such E boxes [2,6].

The role of Myc family proteins in neurodegenerative disorders is poorly understood. In this manuscript, we propose a hypothesis involving a novel neurotoxic pathway that includes expression and phosphorylation of N-Myc by hypoxic induction of HMGA1a, leading to neuronal cell death via induction of PS2V expression.

In brief, human neuroblastoma SK-N-SH and human HEK293T cells were cultured in α-minimal essential and Dulbecco's modified Eagle's medium supplemented with fetal calf serum, respectively. When cells achieved confluence in 175 cm^2 culture plate, the medium was exchanged with serum-free medium. After 4 h, cultures were exposed to hypoxia for 0–21 h using an incubator equipped with low oxygen tension (8 Torr within 3–5 h after cultures were transferred to the hypoxia chamber).

The nuclear fraction was prepared as previously described [38] with minor modifications [18,44,45]. In brief, samples were homogenized in 50 volumes of 10 mM HEPES-NaOH buffer (pH 7.9) containing 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 5 mM dithiothreitol, 10 mM NaF, 10 mM sodium β-glycerophosphate (NaGP), 10 mM sodium pyrophosphate 1 mM sodium orthovanadate (OV), and 1 μg/ml of PMSF at 2 °C. Following the addition of 10% Nonidet P-40 to make a final concentration of 0.6%, homogenates were centrifuged at 15,000 × *g* for 5 min to obtain nuclear fractions. Pellets thus obtained were suspended in 5–10 volumes of 20 mM Tris–HCl buffer (pH 7.5) containing 1 mM EDTA, 1 mM EGTA, 10 mM NaF, 10 mM NaGP, 10 mM sodium pyrophosphate 1 mM OV and 1 μg/ml of PMSF. Immunoblotting was performed as previously described [21,22].

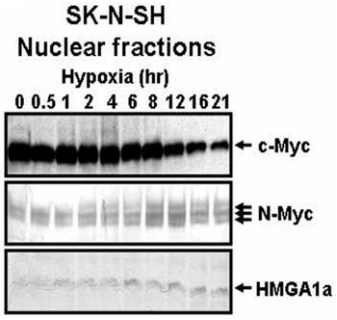

Fig. 1. Effects of hypoxia stimulation on expression of Myc family proteins in human neuroblastoma SK-N-SH cells. Nuclear fractions from SK-N-SH cells were prepared after 0–21 h hypoxia stimulation, followed by immunoblotting assay using anti-c-Myc (top), anti-N-Myc (middle) and anti-HMGA1a (bottom) antibodies.

Nuclear fractions from SK-N-SH cells were obtained after hypoxic stimulation for 0–21 h. These fractions were then subjected to SDS–PAGE and immunoblotting analysis. No significant increase in immunoreactivity was found using an antibody against c-Myc protein (see the corresponding molecular weight position indicated by the black arrow in Fig. 3a and the upper panel of Fig. 1), but PS2V was induced under hypoxic conditions (Fig. 3b) [19,35,36]. On the other hand, exposure to hypoxia for 6 h significantly potentiated N-Myc expression in SK-N-SH cells, with about a 1.5-fold potentiation 8–16 h after exposure to hypoxia (Fig. 3a and the middle panel of Fig. 1b). This potentiation persisted for at least 21 h, after which no further measurements were made (Fig. 3a and the middle panel of Fig. 1b). In contrast, hypoxia did not significantly potentiate HMGA1a expression in SK-N-SH cells following 8 h or less of hypoxic stimulation (Fig. 3a and the middle panel of Fig. 1c). However, increased hypoxic exposure for 8–21 h did lead to stimulation-time dependent expression of HMGA1a in the nuclei of SK-N-SH cells, as observed for N-Myc induction (Fig. 3a and the middle panel of Fig. 1c).

Exposure to hypoxia for 0.5–21 h, under which conditions PS2V expression was not induced, did not significantly alter N-Myc expression in HEK-293T cells (Fig. 3a and the middle panel of Fig. 2). In contrast, hypoxia significantly potentiated c-Myc expression in HEK-293T cells following 1–12 h of hypoxic stimulation, with a decline to control levels thereafter (Fig. 3a and the upper panel of Fig. 2). On the other hand,

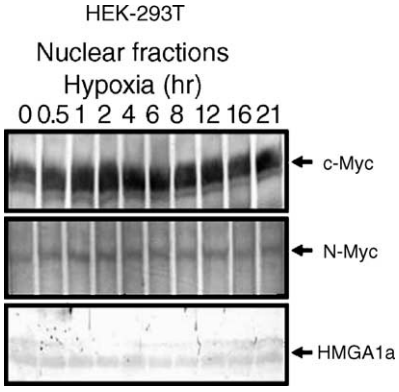

Fig. 2. Effects of hypoxia stimulation on expression of Myc family proteins in HEK-293T cells. Nuclear fractions from HEK-293T cells were prepared after 0–21 h hypoxia stimulation, followed by immunoblotting assay using anti-c-Myc (top), anti-N-Myc (middle) or anti-HMGA1a (bottom) antibodies.

no significant hypoxia-induced expression of HMGA1a was seen in the nuclear fraction from HEK-293T cells (Fig. 3a and the lower panel of Fig. 2). Hence, the hypoxia-induced expression patterns of these proteins differed between SK-N-SH cells and HEK-293T cells (Fig. 3a).

As shown in Fig. 1, hypoxia in SK-N-SH cells potentiated not only N-Myc expression but also additional bands of a higher molecular weight than normal N-Myc (Fig. 4a). Therefore, we investigated whether the high mobility bands produced by hypoxia were due to phosphorylation of the N-Myc protein. Pretreatment with alkaline phosphatase (AP) eliminated the high mobility bands in the nuclear fraction from hypoxia-exposed SK-N-SH cells (Fig. 4b, highest arrow), presumably due to dephosphorylation, and resulted in a large increase in the immunoreactivity of the lowest molecular weight band (Fig. 4b, lowest arrow). Then, the binding activity of N-Myc to its target region in HMGA1a promoter was analyzed by gel retardation shift asaay using the nuclear extracts from normoxia (lane 1), hypoxia (8 h, lane 2) and hypoxia (8 h) + pretreatment with AP (lane 3) (Fig. 4c). The increased binding activity by hypoxia (lane 2) was disappeared in the AP-pretreated nucrear extracts (Fig. 4c, lane 3). Therefore, these results suggest that the reinforced N-Myc expression and phosphorylation by hypoxia lead to activation of HMGA1a transcription. Given these results, nuclear fractions were prepared from SK-N-SH cells after 8 h of hypoxic stimulation, followed by immunoprecipitation with the anti-N-Myc antibody, SDS–PAGE and immunoblotting using an-

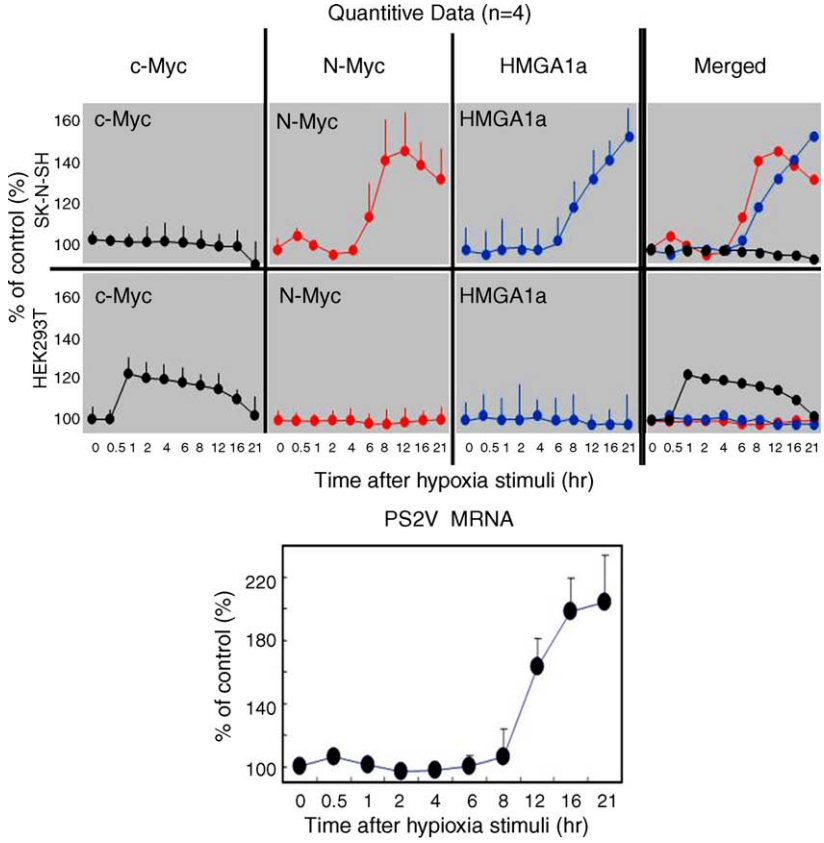

Fig. 3. (a) Quantitative data of Figs. 1 and 2. Data are mean ± S.E. ($n = 3$). c-Myc, N-Myc, HMGA1a and all-merged data of Fig. 1 (upper) and Fig. 2 (lower) were shown. (b) Effects of hypoxia stimulation on expression of PS2V mRNA in SK-N-SH cells. Total RNA from SK-N-SH cells were prepared after 0–21 h hypoxia stimulation, followed by RT-PCR assay as described previously [18,36]. Quantitative data was shown. Data are mean ± S.E. ($n = 3$).

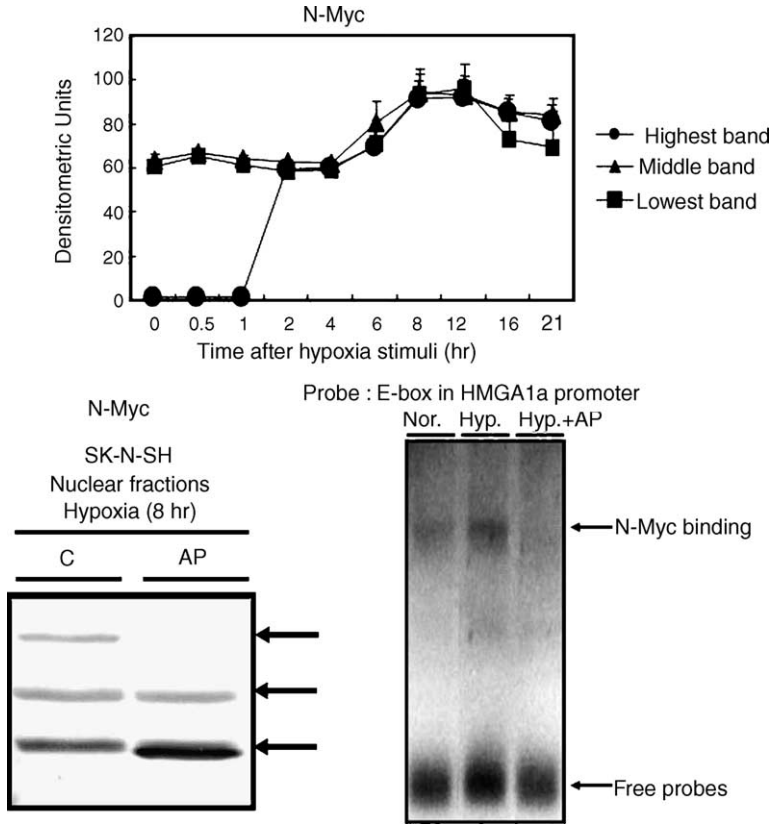

Fig. 4. Effects of alkaline phosphatase on appearance of immunoreactive N-Myc proteins by hypoxia in nuclear fractions from SK-N-SH cells. (a) Individual quantitative data of three different bands in N-Myc were shown. Data are mean ± S.E. ($n = 3$). (b) SK-N-SH cells were stimulated for 8 h under hypoxia, followed by preparation of nuclear fractions. Samples were treated with or without alkaline phosphatase and a subsequent immunoblotting assay using an anti-N-Myc antibody. (c) Effects of alkaline phosphatase (AP) treatment on N-Myc binding with the E-box of HMGA1a promoter in each nuclear extract. Cells were incubated under the normoxic or hypoxic condition for 8 h, followed by collection and preparation of nucrear extracts. AP-treated or untreated samples were subjected to gel retardation electrophoresis and subsequently autoradiography.

tibodies against phosphotyrosine (PY, Fig. 5a), phosphothreonine (PT, Fig. 5b) and phosphoserine (PS, Fig. 5c) to detect possible phosphorylation of N-Myc. Three immunoreactive bands against anti-PS antibody were detected in immunoprecipitates that co-precipitated with anti-N-Myc antibody (Fig. 5c, left panel) after hypoxic stimulation for 8 h (Fig. 5c). Strong immunoreactivity to antibodies against PY (Fig. 5a) and PT (Fig. 5b) was also observed in immunoprecipitates obtained after hypoxic stimulation for 8 h.

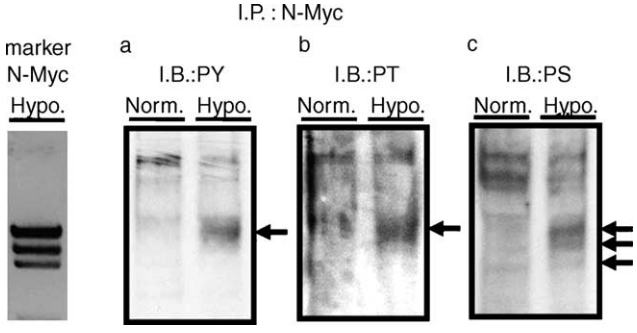

Fig. 5. Effects of hypoxia on phosphorylation of immunoreactive N-Myc protein in nuclear fractions of SK-N-SH cells. Samples were immunoprecipitated with an anti-N-Myc antibody following an immunoblotting assay using anti-phosphotyrosine (a; PY), anti-phosphothreonine (b; PT) or anti-phosphoserine (c; PS) antibodies.

The primary importance of the present findings is that hypoxic stimulation led to differential expression of particular Myc family proteins in nuclear fractions of cell lines, and that this expression occurred in a manner dependent on the hypoxia exposure time. Our previous data showed that exposure to hypoxia for 10–18 h does not induce any significant changes in the expression of HMGA1a protein, HMGA1a mRNA, and PS2V mRNA in non-neuronal cell lines, including HEK293T and HeLa cells, compared with normoxia [19]. The findings in the present study clearly demonstrate that exposure to 0.5–21 h of hypoxia does not induce N-Myc and HMGA1a expression in nuclear fractions from 293T cells (Fig. 2, lower and middle panels). However, transient induction of c-Myc was observed in nuclear fractions from 293T cells (Fig. 2, upper panel). These data suggest that HMGA1a induction by hypoxia does not depend on c-Myc expression, although it has been shown that the HMGA1a gene is directly targeted by c-Myc under other conditions [43].

There are two possible explanations for this inconsistency. One involves participation of the N-Myc protein. Considering that hypoxic stimulation of SK-N-SH cells was performed under PS2V-inducible conditions (Fig. 3b) [19,35,36] (i.e., HMGA1a-inducible conditions [19]), the expression of N-Myc and HMGA1a proteins is in good temporal agreement. That is, the appearance of HMGA1a and PS2V may follow that of N-Myc and HMGA1a, respectively (Fig. 2, lower and middle panels; Fig. 3a, lower and middle panels; Fig. 3b). A second possibility is that under hypoxic conditions there may be differences in the expression and phosphorylation state of Max, which forms a heterodimer with Myc, as well as differences in the phosphorylation state of Myc itself. Both N-Myc and c-Myc form a heterodimer with Max, and this complex can bind to the E-box [2,4,6,30], and regulate HMGA1a [43]. Moreover, many phosphorylation sites on these proteins are known, and these functions are intricately regulated by phosphorylation [9–12,14–17,26,27,33,34,40,42]. It is unclear which functions are required under hypoxia, but precise functional regulation through expression and/or phosphorylation of Max and the level of post-translational modification of Myc family members may lead to PS2V expression in neuroblastoma cell lines such as SK-N-SH cells, but not in 293T and HeLa cells. We note that hypoxia stimulation of SK-N-SH cells led to changes in the phosphorylation of N-Myc, which strongly supports this hypothesis.

As mentioned above, there is accumulating evidence that phosphorylation of the N-Myc protein at serine and/or threonine residues indeed occurs in response to a variety of intracellular signals [10,17]. Furthermore, phosphorylation of the N-Myc protein at Ser-51 by MAP kinase is required for the transcriptional repression activity of the protein [17]. However, the present immunoprecipitation analysis clearly demonstrated phosphorylation of the N-Myc protein at tyrosine residues after 8 h of hypoxia (Fig. 5a). Although the exact molecular mechanism and functional significance are not yet clear, this is the first direct demonstration of phosphorylation of tyrosine residues of N-Myc in nuclear fractions, in response to a particular extracellular signal. N-Myc is a 456-amino acid protein that contains five tyrosine residues at positions 21, 28, 324, 330 and 421. Serine and threonine phosphorylation in N-Myc is more likely than tyrosine phosphorylation in the initial response to hypoxia. However, phosphorylation of tyrosines, as well as of serines and threonines, could play a crucial role underlying subsequent consolidation of extracellular signals due to hypoxia, through modulation of the transcriptional activity of the Myc/Max complex in neuroblastoma cell lines. However, phosphorylation of Myc family members other than N-Myc was not confirmed in the present study.

In conclusion, our results suggest that differences in expression and in the phosphorylation state of Myc proteins occur in response to a hypoxic stimulus, and that these in part cause induction of HMGA1a and subsequent generation of PS2V, which in turn has aberrant effects on the nervous system.

References

[1] Y. Azuma, K. Ogita, Y. Yoneda, Particular nuclear transcription factors responsive to systemic administration of kainic acid in murine brain, Neurochem. Int. 29 (1996) 289–299.

[2] N. Benvenisty, D.M. Ornitz, G.L. Bennett, B.G. Sahagan, A. Kuo, R.D. Cardiff, P. Leder, Brain tumours and lymphomas in transgenic mice that carry HTLV-I LTR/c-myc and Ig/tax genes, Oncogene 7 (1992) 2399–2405.

[3] O. Bernard, J. Drago, H. Sheng, L-myc and N-myc influence lineage determination in the central nervous system, Neuron 9 (1992) 1217–1224.

[4] E. Blackwood, R.N. Eisenman, Max: a helix–loop–helix zipper protein that forms a sequence-specific DNA-binding complex with Myc, Science 251 (1991) 1211–1217.

[5] M.D. Cole, S.B. McMahon, The Myc oncoprotein: a critical evaluation of transactivation and target gene regulation, Oncogene 18 (1999) 2916–2924.

[6] M. Eilers, S. Schirm, J.M. Bishop, The MYC protein activates transcription of the alpha-prothymosin gene, EMBO J 10 (1991) 133–141.

[7] I. Ferrer, R. Blanco, N-myc and c-myc expression in Alzheimer disease, Huntington disease and Parkinson disease, Brain Res. Mol. Brain Res. 77 (2000) 270–276.

[8] I. Ferrer, R. Blanco, M. Carmona, B. Puig, Phosphorylated c-MYC expression in Alzheimer disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration, Neuropathol. Appl. Neurobiol. 27 (2001) 343–351.

[9] S. Gupta, A. Seth, R.J. Davis, Transactivation of gene expression by Myc is inhibited by mutation at the phosphorylation sites Thr-58 and Ser-62, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 3216–3220.

[10] T. Hagiwara, K. Nakaya, Y. Nakamura, H. Nakajima, S. Nishimura, Y. Taya, Specific phosphorylation of the acidic central region of the N-myc protein by casein kinase II, Eur. J. Biochem. 209 (1992) 945–950.

[11] M. Henriksson, A. Bakardjiev, G. Klein, B. Luscher, Phosphorylation sites mapping in the N-terminal domain of c-myc modulate its transforming potential, Oncogene 8 (1993) 3199–3209.

[12] A.T. Hoang, B. Lutterbach, B.C. Lewis, T. Yano, T.Y. Chou, J.F. Barrett, M. Raffeld, S.R. Hann, C.V. Dang, A link between increased transforming activity of lymphoma-derived MYC mutant alleles, their defective regulation by p107, and altered phosphorylation of the c-Myc transactivation domain, Mol. Cell. Biol. 15 (1995) 4031–4042.

[13] B. Hoffman, D.A. Liebermann, The proto-oncogene c-myc and apoptosis, Oncogene 17 (1998) 3351–3357.

[14] B. Lutterbach, S.R. Hann, Hierarchical phosphorylation at N-terminal transformation-sensitive sites in c-Myc protein is regulated by mitogens and in mitosis, Mol. Cell. Biol. 14 (1994) 5510–5522.

[15] B. Lutterbach, S.R. Hann, Overexpression of c-Myc and cell immortalization alters c-Myc phosphorylation, Oncogene 14 (1997) 967–975.

[16] B. Lutterbach, S.R. Hann, c-Myc transactivation domain-associated kinases: questionable role for map kinases in c-Myc phosphorylation, J. Cell Biochem. 72 (1999) 483–491.

[17] A. Manabe, S.M. Iguchi-Ariga, H. Iizuka, H. Ariga, Transcriptional repression activity of N-MYC protein requires phosphorylation by MAP kinase, Biochem. Biophys. Res. Commun. 219 (1996) 813–823.

[18] T. Manabe, T. Kitayama, K. Ogita, Y. Yoneda, Differential expression and phosphorylation of particular Fos family members by kainate in nuclear and cytosolic fractions of murine hippocampus, Neuroscience 100 (2000) 453–463.

[19] T. Manabe, T. Katayama, N. Sato, F. Gomi, J. Hitomi, T. Ynagita, T. Kudo, A. Honda, Y. Mori, K. Imaizumi, A. Mayeda, M. Tohyama, Induced HMGA1a expression causes aberrant splicing of presenilin-

2 pre-mRNA in sporadic Alzheimer's disease, Cell Death Differ. 10 (2003) 698–708.
[20] T. Manabe, T. Katayama, N. Sato, T. Kudo, S. Matsuzaki, K. Imaizumi, M. Tohyama, The cytosolic inclusion bodies that consists of splice variants that lack exon 5 of the presenilin-2 gene differ obviously from Hrano bodies observed in the brain from sporadic cases of Alzheimer's disease patients, Neurosci. Lett. 323 (2002) 198–200.
[21] T. Manabe, N. Kuramoto, N. Nakamichi, K. Aramachi, K. Baba, T. Hirai, M. Yoneyama, Y. Yoneda, Degradation of c-Fos protein expressed by *N*-methyl-D-aspartic acid in nuclear fractions of murine hippocampus., Brain Res. 905 (2001) 34–43.
[22] T. Manabe, K. Ogita, N. Nakamichi, Y. Yoneda, Differential in vitro degradation of particular Fos family members expressed by kainic acid in nuclear and cytosolic fractions of murine hippocampus, J. Neurosci. Res. 64 (2001) 34–42.
[23] L. McGahan, A.M. Hakim, G.S. Robertson, Hippocampal Myc and p53 expression following transient global ischemia, Brain Res. Mol. Brain Res. 56 (1998) 133–145.
[24] T. Nakagomi, A. Asai, H. Kanemitsu, K. Narita, Y. Kuchino, A. Tamura, T. Kirino, Up-regulation of c-myc gene expression following focal ischemia in the rat brain, Neurol. Res. 18 (1996) 559–563.
[25] A. Nisikawa, T. Manabe, T. Katayama, T. Kudo, S. Matsuzaki, T. Yanagita, H. Okuda, Y. Bando, M. Tohyama, Novel function of PS2V: change in conformation of tau proteins, Biochem. Biophys. Res. Commun. 318 (2004) 435–438.
[26] K. Noguchi, C. Kitanaka, H. Yamana, A. Kokubu, T. Mochizuki, Y. Kuchino, Regulation of c-Myc through phosphorylation at Ser-62 and Ser-71 by c-Jun N-terminal kinase, J. Biol. Chem. 274 (1999) 32580–32587.
[27] N. Nozaki, T. Naoe, T. Okazaki, Immunoaffinity purification and characterization of CACGTG sequence-binding proteins from cultured mammalian cells using an anti c-Myc monoclonal antibody recognizing the DNA-binding domain, J. Biochem. (Tokyo) 121 (1997) 550–559.
[28] A.J. Obaya, M.K. Mateyak, J.M. Sedivy, Mysterious liaisons: the relationship between c-Myc and the cell cycle, Oncogene 18 (1999) 2934–2941.
[29] G.C. Prendergast, Mechanisms of apoptosis by c-Myc, Oncogene 18 (1999) 2967–2987.
[30] G.C. Prendergast, D. Lawe, E.B. Ziff, Association of Myn, the murine homolog of Max with c-Myc stiumulates methylation-sensitive DNA binding and Ras cotransformation, Cell 65 (1991) 395–407.
[31] R. Reeves, Molecular biology of HMGA proteins: hubs of nuclear function, Gene 277 (2001) 63–81.
[32] J. Rossler, M. Schwab, W. Havers, L. Schweigerer, Hypoxia promotes apoptosis of human neuroblastoma cell lines with enhanced N-myc expression, Biochem. Biophys. Res. Commun. 281 (2001) 272–276.
[33] K. Saksela, T.P. Makela, G. Evan, K. Alitalo, Rapid phosphorylation of the L-myc protein induced by phorbol ester tumor promoters and serum, EMBO J. 8 (1989) 149–157.
[34] K. Saksela, T.P. Makela, K. Hughes, J.R. Woodgett, K. Alitalo, Activation of protein kinase C increases phosphorylation of the L-myc trans-activator domain at a GSK-3 target site, Oncogene 7 (1992) 347–353.
[35] N. Sato, O. Hori, A. Yamaguchi, J.C. Lambert, M.C. Chartier-Harlin, P.A. Robinson, A. Delacourte, A.M. Schmidt, T. Furuyama, K. Imaizumi, M. Tohyama, T. Takagi, A novel presenilin-2 splice variant in human Alzheimer's disease brain tissue, J. Neurochem. 72 (1999) 2498–2505.
[36] N. Sato, K. Imaizumi, T. Manabe, M. Taniguchi, J. Hitomi, T. Katayama, T. Yoneda, T. Morihara, Y. Yasuda, T. Takagi, T. Kudo, T. Tsuda, Y. Itoyama, T. Makifuchi, P.E. Fraser, P. St George-Hyslop, M. Tohyama, Increased production of b-amyloid and vulnerability to ER stress by an aberrant spliced form of presenilin-2, J. Biol. Chem. 276 (2001) 2108–2114.
[37] S. Sawai, A. Shimono, Y. Wakamatsu, C. Palmes, K. Hanaoka, H. Kondoh, Defects of embryonic organogenesis resulting from targeted disruption of the N-myc gene in the mouse, Development 117 (1993) 1445–1455.
[38] E. Schreiber, P. Matthias, M.M. Muller, W. Schaffner, Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells, Nucleic Acids Res. 17 (1989) 6419.
[39] D.J. Selkoe, Normal and abnormal biology of the beta-amyloid precursor protein, Annu. Rev. Neurosci. 17 (1994) 489–517.
[40] A. Seth, E. Alvarez, S. Gupta, R.J. Davis, A phosphorylation site located in the NH2-terminal domain of c-Myc increases transactivation of gene expression, J. Biol. Chem. 266 (1991) 23521–23524.
[41] Y. Wakamatsu, Y. Watanabe, H. Nakamura, H. Kondoh, Regulation of the neural crest cell fate by N-myc: promotion of ventral migration and neuronal differentiation, Development 124 (1997) 1953–1962.
[42] A. Wenzel, C. Cziepluch, U. Hamann, J. Schurmann, M. Schwab, The N-Myc oncoprotein is associated in vivo with the phosphoprotein Max(p20/22) in human neuroblastoma cells, EMBO J. 10 (1991) 3703–3712.
[43] L.J. Wood, M. Mukherjee, C.E. Dolde, Y. Xu, J.F. Maher, T.E. Bunton, J.B. Williams, L.M. Resar, HMG-I/Y, a new c-Myc target gene and potential oncogene, Mol. Cell. Biol. 20 (2000) 5490–5502.
[44] Y. Yoneda, K. Ogita, Y. Azuma, M. Ikeda, H. Tagami, T. Manabe, *N*-Methyl-D-aspartate signaling to nuclear activator protein-1 through mechanisms different from those for kainate signaling in murine brain, Neuroscience 90 (1999) 519–533.
[45] Y. Yoneda, K. Ogita, Y. Azuma, N. Kuramoto, T. Manabe, T. Kitayama, Predominant expression of nuclear activator protein-1 complex with DNA binding activity following systemic administration of *N*-methyl-D-aspartate in dentate granule cells of murine hippocampus, Neuroscience 93 (1999) 19–31.