

p53 and Cell Cycle Proteins Participate in Spinal Motor Neuron Cell Death in ALS

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Abstract: Apoptosis has been implicated in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). We previously demonstrated a role for G1 to S phase cell cycle regulators in ALS with increased levels of hyperphosphorylated retinoblastoma (ppRb) and E2F-1 in ALS spinal cord motor neurons. In this study we examined the levels of the cell cycle checkpoint tumor suppressor protein p53 with concurrent changes in cell death markers during ALS. Expression and subcellular distribution of p53, retinoblastoma, Bax, Fas, and caspases were explored by immunoblot, immunohistochemistry and double-label confocal microscopy in the spinal cord and motor cortex of ALS and control subjects. We identified elevated levels of p53 in ALS spinal cord motor neurons but not neurons in the motor cortex. In addition, there was an increase in Bax, Fas, caspases-8 and -3 proteins in ALS spinal motor neurons. While caspase-3 and TUNEL labeled neurons were positive for ppRb, E2F-1 and p53 in spinal motor neurons, and Fas co-localized with caspase-8 in spinal motor neurons, we failed to observe these results in large neurons in the motor cortex of ALS subjects. We have linked p53 and activation of G1 to S phase cell cycle regulators to an apoptotic mode of cell death ALS spinal cord motor neurons.

Keywords: Amyotrophic lateral sclerosis, p53, retinoblastoma, caspase.

INTRODUCTION

The control of cell proliferation or growth arrest is intimately linked to the control of programmed cell death or apoptosis. Cell cycle components such as ppRb, E2F and p53 function in both cell cycle progression and apoptosis [1-3]. In fact the promoters of p53, p73, human cytochrome c1 and apoptotic protease activating factor 1 (APAF1) contain E2F-1 binding sites suggesting an E2F-1-mediated transcriptional regulation [3-6]. β -amyloid-mediated toxicity in PC12 cells was found to be mediated by a E2F-1 - p53 - Bax pathway and the over-expression of E2F-1 in these cells led to DNA fragmentation in addition to the induction of p53 (by E2F-1) and Bax (by p53) [7]. These studies directly link E2F-1 to p53 and apoptosis.

In models of acute and chronic neurological disease, a role for aberrant cell cycle activation and subsequent neuronal apoptosis has been established. These studies include cell death mediated by β -amyloid, presenilin-1, DNA damage, oxidative stress, membrane depolarization, and 6-hydroxydopamine (6-OHDA) [8-12]. DNA damage induced phosphorylation of p53 has been shown to activate cell cycle proteins leading to neuronal cell death [13]. Inhibition of p53 through the use of antisense and dominant negative constructs protects neurons from this wide array of toxic insults [14, 15].

Motor neurons of ALS patients exhibit characteristics of apoptosis in post-mortem tissues [16], and apoptosis is believed to be the mode of cell death in ALS [17]. Increased levels of p53 with concomitant elevation in DNA binding activity has been demonstrated in motor neurons and astroglia of the affected regions of ALS patients but not in controls [18]. This study was corroborated in ALS transgenic mice over-expressing mutant G86R SOD1 protein [19]. Defective DNA repair and enhanced DNA damage are also evident in ALS motor neurons [20-23]. Cell death induced by DNA damage of cultured motor neurons has been linked to Fas and p53 activation [24]. Increased p53 gene expression has been observed in degenerative spinal cords from the wobbler mouse model and in the ventral horns of ALS patients [25].

In the current study we examined the expression and distribution of p53, markers of apoptosis and G1 to S phase cell cycle regulators. We hypothesized that alterations in the cell cycle regulator, p53, will result in concurrent changes in cell cycle proteins and apoptotic death markers during ALS. We present evidence for increased accumulation of p53 in the nucleus of ALS spinal cord motor neurons but not neurons of the motor cortex. Laser scanning confocal microscopy demonstrates that the G1 to S phase cell cycle regulators co-localize with markers of apoptosis (TUNEL and Caspase-3) and p53. Taken together, these results suggest a role for p53 and caspase activation in conjunction with aberrant re-activation of cell cycle regulators during sporadic ALS (SALS).

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MATERIALS AND METHODOLOGY

Source of Tissue Samples

The lumbar spinal cord and pre/post-central gyrus (motor/sensory) region from eighteen cases of clinically diagnosed (using El Escorial criteria) and neuropathologically confirmed sporadic ALS (SALS), and ten non-ALS age-matched controls, were utilized to examine protein expression and distribution as previously detailed [26]. All autopsy tissues were obtained from the University of Pittsburgh ALS Tissue Bank. Human tissue samples were obtained at the time of autopsy using University of Pittsburgh Internal Review Board approved consent forms. Approval for use of autopsy human tissues was obtained from the University of Pittsburgh Committee for Oversight of Research Involving the Dead (CORID). Neuropathologic assessment was used to confirm the clinical diagnosis of each subject.

Antibodies

Monoclonal antibodies were used to detect E2F-1 (KH95; Santa Cruz Biotechnology, Santa Cruz, CA, USA), p53 (DO-1; Santa Cruz), and Fas (B10; Santa Cruz). Polyclonal antibodies were used to detect ppRb (NEN Biolabs, Ipswich, MA), actin (Millipore, Billerica, MA, USA), BAX (BD Biosciences, San Jose, CA, USA), caspase-3 (Cell Signaling Technology, Danvers, MA, USA), caspase-8 (Biomedica Corp, Foster City, CA), p73 (H79; Santa Cruz). The antibody concentrations for immunoblotting were 1:600 (caspase-8), 1:700 (caspase-3), 1:750 (p53, p73, Fas), 1:1000 (BAX) and 1:1500 (Actin). For light microscopy, these antibodies were used at dilutions of 1:100 (caspase-8), 1:150 (p53, caspase-3, Fas), 1:200 (BAX). For confocal laser scanning microscopy, we used primary antibody concentrations at 1:80 (ppRb, p53, caspase-3, caspase-8, Fas), and 1:60 (E2F-1).

Light and Confocal Microscopy

Paraffin-embedded tissue sections from lumbar or cervical spinal cord and mid-motor cortical regions were used as per published protocols [26]. The dorsal horn of the spinal cord and sensory cortex were the internal controls for immunohistochemistry. The study was conducted in a blinded fashion using eighteen ALS and ten age-matched control cases.

For laser scanning confocal microscopy, tissues were processed as for light microscopy with the following modifications. Primary antibodies were used at a higher concentration (ppRb; and the slides incubated overnight at 4°C. After incubations with biotinylated secondary antibodies (1:1000), the signal was further amplified using the tyramide amplification kit as per manufacturer's protocol. The sections were finally incubated with a 1:500 dilution of streptavidin-Alexa Green (green) or streptavidin-Cy5/Cy3 (red) at a dilution of 1:500. A total of 70-80 and 20-30 motor neurons in the spinal cord and motor cortex, respectively, were counted at 400X magnification. The confocal study was conducted in a blinded fashion. Single labeling and other control experiments were performed to determine optimal concentrations of primary and fluorescently labeled secondary antibodies. Omission of the primary antibody resulted in absence of fluorescent signal.

Crossover control experiments were also performed to determine the specificity of the secondary antibody.

Protein Extraction

Lumbar spinal cord and motor cortical (mid- and superior-motor cortical regions) frozen tissue from controls and ALS cases were utilized for immunoblotting and DNA binding assays. For total cell lysates, tissue samples were homogenized using polytron homogenizer (PGC Scientific, Gaithersburg, MD) set at 15000 rpm for 45 secs. It was carried out in lysis buffer containing 25mmol/L HEPES (pH 7.4), 50mmol/L NaCl, protease inhibitor cocktail II (SIGMA), and 1% triton-X 100. The homogenized product was spun at 14000 rpm in a cold microfuge and the supernatant saved as the total cell lysate. Nuclear and post-nuclear extracts were extracted as described previously [26]. Briefly, protein extracts were prepared by detergent lysis on ice (0.1% Nonidet P-40, 10 mM Tris (pH 8.0), 10 mM MgCl₂, 15 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 2 µg/ml pepstatin A, and 1 µg/ml leupeptin). The nuclei were collected by low speed centrifugation at 800x g for 5 mins. The supernatant was saved as the post-nuclear supernatant and the pellet containing the nuclei was further extracted with high salt buffer (0.42 mol/l NaCl, 20 mmol/L HEPES (pH 7.9), 20% glycerol, 0.5 mmol/L phenylmethylsulfonyl fluoride, 2 µg/ml pepstatin A, and 1 µg/ml leupeptin) on ice for 10 mins. Residual insoluble material was removed by centrifugation at 14,000 x g for 5 mins. The resulting supernatant fraction was collected and termed the "nuclear extract". Protein concentrations were determined by Bio-Rad assay (Bio-Rad, Richmond, CA, USA).

Immunoblotting

Total cell lysates, nuclear extracts and post-nuclear supernatants were fractionated by electrophoresis on an 8, 10 or 12% sodium dodecyl sulfate-polyacrylamide gels. The proteins were transferred to polyvinylidene difluoride nylon membranes (NEN Biolabs) and blocked in 5% nonfat milk/1X PBS or 0.5% BSA / 0.15% glycine in 1X PBS overnight at 4°C. The blots were probed individually with the primary antibodies overnight at 4°C in 0.5% milk/PBS. The blots were washed three times in PBS/0.1% Tween-20 for 15 minutes. Isotype-specific horseradish peroxidase-conjugated secondary antibodies (Chemicon; goat anti-mouse; 1:2000, goat anti-rabbit; 1:2000) were added for 2 hrs at room temperature. The secondary antibodies were washed extensively in PBS / 0.1% Tween-20 (three times for 20 mins). The final reaction products were visualized using enhanced chemiluminescence (ECL; Pierce) and the band intensities were within the linear range of detection. Actin was used to normalize protein levels within each sample. The density of bands was measured using the NIH Image software version 1.58 (National Institutes of Health, USA).

Electrophoretic Mobility Shift Assay (EMSA)

Gel mobility shift assays were performed as described earlier [26] with the following modifications. The sequences used to probe for p53 specific DNA binding activity were: WT p53 5'-TACAGAACATGTCTAAGCATGCTGGGGA CT -3'; MT p53 5'- TACAGAATCGCTCTAAGCATGCT GGGACT -3'. For competition reactions, unlabeled p53

Fig. (6). E2F-1 and p53 co-localize with caspase-3 in ALS spinal motor neurons. Tissue sections of lumbar spinal cord (A and B) and motor cortex (C and D) from 11 ALS and 6 age-matched controls were labeled with the combinations of p53 or E2F-1 (Alexa green conjugated secondary antibody) and active caspase-3 (Cy5 conjugated secondary antibody). Arrows in the control panels indicate representative motor neurons. E2F-1 and p53 co-localize with caspase-3 in spinal motor neurons of ALS patients. All panels are at X400 magnification.

approximately 25% of the ppRb positive motor neurons and 30% of p53 positive motor neurons had fragmented DNA detected by TUNEL (arrowheads in Fig. 8A, B, respectively). We also identified ppRb or p53 positive but TUNEL negative ALS motor neurons (asterisk in Fig. 8A inset). Approximately 75% of the spinal motor neurons that exhibited TUNEL also contain nuclear p53. Motor neurons of control subjects lack TUNEL staining (Fig. 8). In the motor cortex, approximately 64% of the large pyramidal neurons were positive for ppRb (Fig. 8C, arrow) while only 9% were p53 positive (Fig. 8D). There were TUNEL positive small cells in the gray matter of the cortex but not in any large pyramidal neurons (arrows and arrowheads in Figs. 8C, D).

DNA-Binding Activity of p53

To determine the functional status of p53 in ALS, DNA binding activity was analyzed using nuclear extracts of spinal cord and motor cortex and radiolabeled oligonucleotide containing the consensus p53 binding site. DNA binding was monitored by gel mobility shift assays (EMSA) (Fig. 9). Competition experiments with excess unlabeled wild-type or mutant oligonucleotide demonstrated the specificity of the complex observed by EMSA (Fig. 9A). The p53 DNA binding activity in the spinal cord or motor cortex exhibited no significant difference between control and ALS (Figs. 9B-D).

DISCUSSION

The balance between pro- and anti-apoptotic proteins modulates cell survival and influences the mode of cell death. Prior studies have suggested that motor neurons enter an apoptotic mode of cell death during ALS [17]. In this study we report altered levels and co-localization of p53 and pro-apoptotic proteins in ALS post-mortem tissues. Interestingly, p53 protein levels were increased in the ALS spinal motor neurons but not neurons of the motor cortex. Fas and caspase-8 immunoreactivity was also evident specifically in spinal motor neurons of ALS patients (Fig. 7).

The role of p53 in ALS has been questioned due to results obtained from transgenic SOD1 mice studies. Loss of p53 in p53^{-/-}/G93A SOD1⁺ double transgenic mice did not alter disease onset or progression [27]. A similar study using transgenic mice containing a low copy number of the G93A SOD1 mutant transgene reported identical results [28]. However SOD1 induced motor neuron cell death may occur *via* p53 independent mechanisms and not mimic conditions in sporadic ALS patients. Depending on the cell type, over-expression of p53 can result in either cell growth arrest or apoptosis [29-32]. Conversely, p53 knockout induces resistance to apoptosis [33, 34]. Thymocytes from these mice are resistant to DNA damaging agents such as ionizing radiation and topoisomerase II inhibitors (etoposide) but not

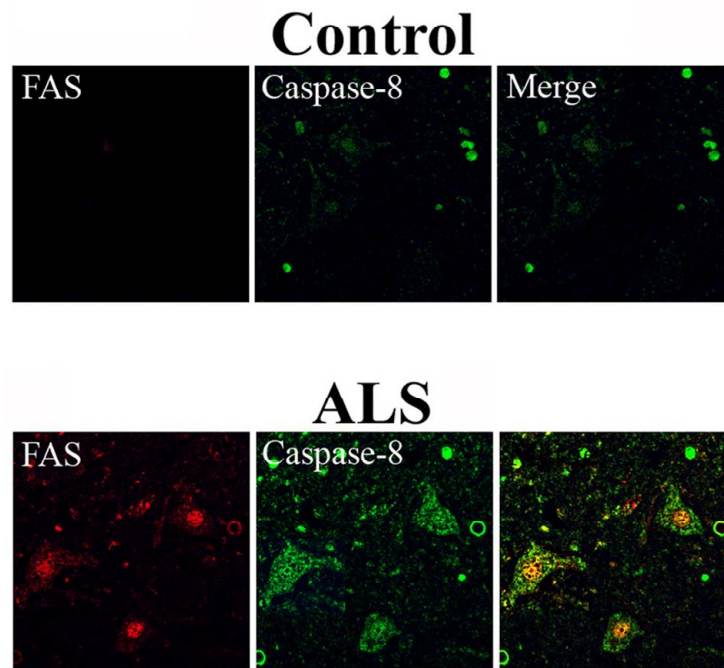


Fig. (7). Fas and Caspase-8 co-localize in spinal motor neurons. Lumbar spinal cord sections from 11 ALS and 6 age-matched controls were labeled with anti-caspase-8 (Alexa green conjugated secondary antibody) and anti-Fas (Cy5 conjugated secondary antibody) antibodies. In ALS motor neurons, Fas (red signal) is present in the nucleus and co-localized with caspase-8.

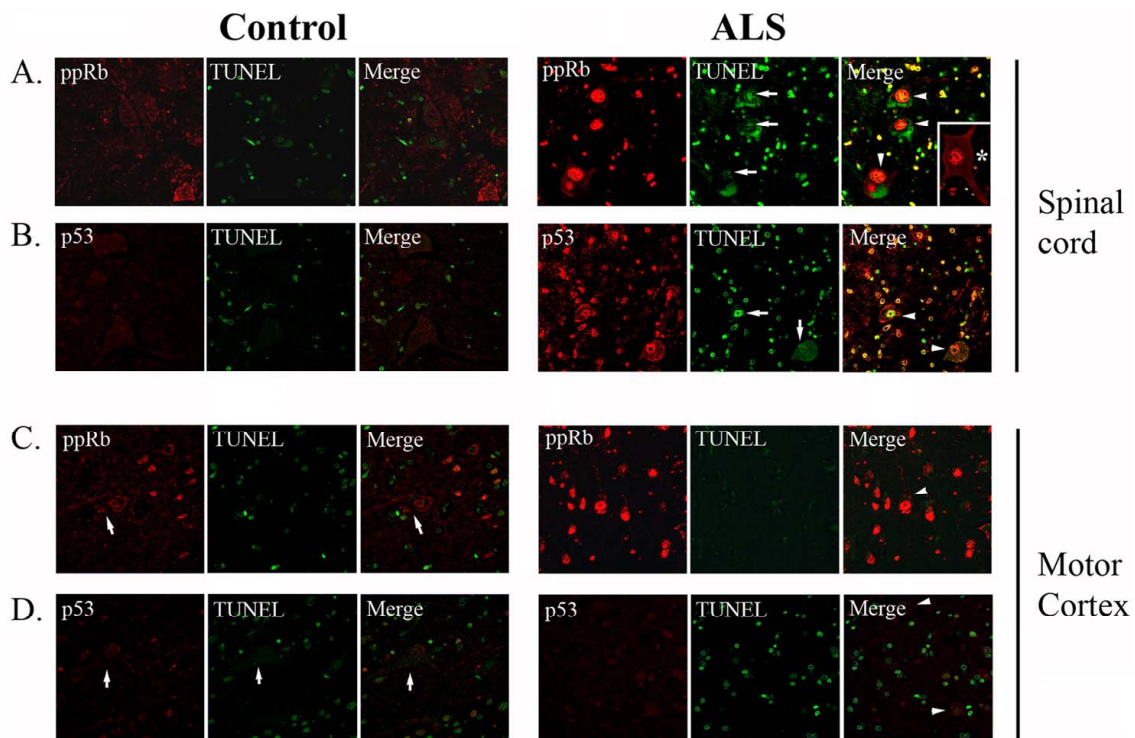


Fig. (8). Increased DNA fragmentation in spinal but not cortical motor neurons. Lumbar spinal cord (**A** and **B**) and motor cortex (**C** and **D**) sections from 11 ALS and 6 age-matched controls were double-labeled for TUNEL (FITC-conjugated) and p53 or ppRb (Cy-5 conjugated). Sections from all control subjects exhibited negligible co-localization of ppRb or p53 and TUNEL within neurons. Arrows in **A** and **B** indicate TUNEL positive spinal motor neurons. TUNEL positive motor neurons were also ppRb or p53 immunoreactive in the spinal cord (Merge panels; arrowheads). The asterisk in the inset depicts a ppRb(+) but TUNEL(-) ALS motor neuron. The cortical motor neurons in ALS cases were ppRb positive (**C**) and p53 negative (**D**). Motor neurons in panels **C** and **D** appear negative for TUNEL (arrows indicate control motor neurons and arrowheads indicate ALS motor neurons).

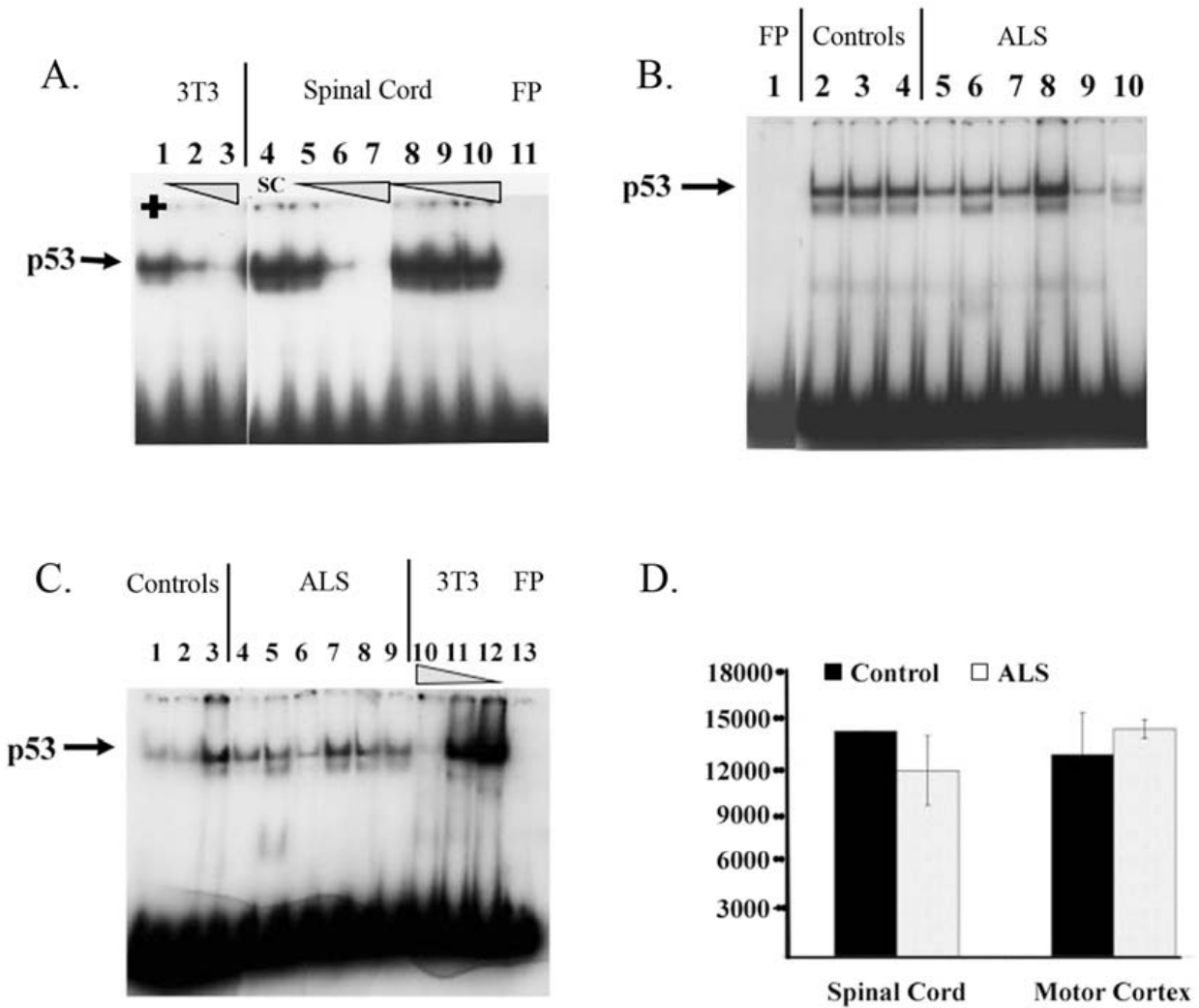


Fig. (9). No change in p53 DNA binding activity in ALS motor neurons. Nuclear extracts from lumbar spinal cord and motor cortex were used for electrophoretic mobility shift assays (EMSA). **(A):** Competition EMSA using radiolabeled p53 oligonucleotide and either extract from NIH3T3 cells as a positive control (lanes 1 to 3) or spinal cord nuclear extracts (25µg; lanes 4 to 7) indicating a concentration dependent competition (lanes 2 – 3 and 5 – 7) when excess cold oligonucleotides were used in the binding reaction. The complex remains unaltered when excess unrelated oligonucleotides (lanes 8 – 10) were utilized in the binding reaction. Lane assignments for competition assays were as follows: Lanes 2 and 3: 3T3 extract with excess unlabeled p53 oligonucleotide (3ng and 100ng, respectively); Lanes 5 to 7: spinal cord extract with excess unlabeled p53 oligonucleotide (3ng, 30ng and 100ng, respectively); Lanes 8 to 10: spinal cord extract with excess unlabeled unrelated oligonucleotide (30ng, 100ng and 300ng, respectively); Lane 11: Free radiolabeled probe alone (FP). **(B):** EMSA of control or ALS spinal cord nuclear extract and radiolabeled p53 oligonucleotide. Lane 1 is free probe alone; Lanes 2 to 4 are Control subjects; Lanes 5 to 10 are ALS cases. **(C):** EMSA of control or ALS motor cortex nuclear extract and radiolabeled p53 oligonucleotide. Lanes 1 to 3 are Control subjects; Lanes 4 to 9 are ALS subjects; Lanes 10 to 12 are 3T3 extracts with 100ng excess unlabeled p53 oligonucleotide (lane 10), 3ng excess unlabeled p53 oligonucleotide (lane 11), and radiolabeled p53 oligonucleotide without competitor (lane 12). Lane 13 is free probe alone. **(D):** Densitometry measurement of the p53:DNA complex in each lane using NIH 1.58 software indicates unaltered levels of DNA binding activity in either the spinal cord or motor cortex. Controls are denoted in black bars (n=3) and ALS cases in stippled bars (n=6). There is no statistically significant alteration in DNA binding activity.

to cell death induced by glucocorticoids or phorbol ester [35]. This suggests that both p53-dependent and independent cell death pathways exist.

We reported an aberrant activation of G1 to S phase regulators in ALS motor neurons marked by altered levels of ppRb and E2F-1 [26]. Our currently study indicates the co-localization of these cell cycle regulators with nuclear p53.

While p53 is negatively regulated by direct protein-protein interactions with modulators such as mdm-2, it is stabilized by the action of CDK inhibitors such as p14^{ARF}. Furthermore, post-translational modifications such as phosphorylation can enhance the transcriptional activity of p53 [36]. Our immunoblot results indicate increased levels of phosphorylated p53 in the nuclear fractions of spinal cord

tissue extracts. In a prior study, protein levels of nuclear p53 and its DNA binding activity were increased in affected CNS regions of ALS patients [18]. However, we found no significant difference in the DNA binding activity of p53 in ALS spinal cord despite its enhanced nuclear localization. This result is likely due to the fact that the nuclear extracts used for EMSA are generated from a heterogeneous cell population within the tissue.

In normal physiologic conditions p53 has a very short half-life and is rapidly degraded. The presence of elevated levels of p53 in ALS patients suggests that the protein is aberrantly stabilized either by the down-regulation of proteins that negatively regulate p53 such as mdm2 or by reduced degradation of p53 *via* the proteasomal machinery. It is well documented that the proteasome machinery is compromised in neurodegenerative diseases such as ALS with concomitant increases in aggregate formations [37-39]. It is possible that p53 transcripts are stabilized in ALS through RNA binding proteins or that post-translational modification of p53 or p53 binding proteins can alter p53 stability and its consequent activity.

The TAR DNA binding protein (TDP-43) is a nuclear ribonucleoprotein (hnRNP) that functions in pre-mRNA splicing, transcription and stability of mRNA [40]. TDP-43 has been localized to cytoplasmic inclusions in ALS and frontotemporal lobar degeneration (FTLD) patients [41]. While transgenic mice expressing mutant human SOD1 fail to exhibit cytoplasmic TDP-43 inclusions (nor altered p53), the wobbler mouse exhibits TDP-43 accumulation and inclusions in the cytoplasm. Loss of TDP-43 increases cyclin-dependent kinase 6 (cdk6) levels, pRb hyperphosphorylation, and apoptosis. We observed increased nuclear ppRb in ALS motor neurons and co-localization of p53 and ppRb (Fig. 5). We propose that p53 participates in cell death induced by TDP-43 accumulation in cytoplasmic inclusions. Further studies are required to examine the link between TDP-43, p53 and motor neuron cell death.

We further investigated the correlation between p53 subcellular distribution and markers of motor neuron cell death. p53 co-localized with activated caspase-3 immunoreactivity and TUNEL staining (Figs. 6, 8). Altered subcellular distribution of p53 could be in response to DNA damage to induce re-entry into the cell cycle. DNA damage can activate proteins that induce cell cycle arrest and/or DNA repair. However, extensive DNA damage induces neuronal apoptosis. The presence of p53 in the nucleus of motor neurons that apparently lack dendritic processes with a rounded cell body suggest that nuclear accumulation of p53 may correspond to cell atrophy and death.

Our p53 data in the spinal cord of ALS patients contrasts that obtained in the motor cortex of these same subjects. Surprisingly, neurons of the motor cortex exhibited negligible p53 immunoreactivity. p53 was undetectable by immunoblot and its DNA binding activity unaffected by ALS. This suggests that motor neuron loss is p53-dependent in spinal cord motor neurons and p53-independent in upper motor neurons of the motor cortex. In addition, studies in adult mice and neuronal cell cultures show evidence for localized transcription-independent mechanism of p53 action in the synapses [42]. Oxidative and excitotoxic insults increased the levels of active (phosphorylated) p53 in

isolated cortical synaptosomes, which preceded the loss of synapsin I. This suggests that p53 can affect the dysfunction and degeneration of synapses independent of modulating gene expression.

Irrespective of the mechanism that induces cell death, apoptosis involves the induction of pro-apoptotic genes with downstream activation of caspases. Our data suggests the activation of apoptosis downstream of cell cycle activation. Both mitochondrial mediated (increased Bax and caspase-3) and death receptor-mediated pathways (increased Fas, caspase-8 and caspase-3) may be involved in lower motor neuron death in ALS. Active caspase-3 immunoreactivity was detected in punctate structures in the cytoplasm and the nucleus of spinal motor neurons. This data is consistent with prior studies in the mutant SOD1 transgenic mouse model of ALS that also implicates caspase induced apoptosis as a mechanism of motor neuron cell death [43, 44]. We also used DNA fragmentation by TUNEL staining as a measure of apoptosis in motor neurons. It is noteworthy to find that there was increased evidence for fragmented DNA in the spinal cord but not the motor cortex. In the spinal cord, the presence of p53 immunoreactivity in 75% of the TUNEL positive motor neurons suggests that DNA damage results in p53 accumulation. However only 30% of the p53 positive neurons were TUNEL positive, suggesting that p53 stabilization/activation may occur *via* other means unrelated to DNA damage. While TUNEL does not differentiate between single-strand (necrosis) and double-strand (apoptosis) DNA breaks, our results do indicate the presence of nicked DNA that co-localizes to the G1 to S phase regulators. These observations coupled with the presence of activated caspase-3 lend further support for an apoptotic mode of cell death in the spinal cord.

Fas-mediated cell death has been reported in cultured motor neurons from mutant SOD1 transgenic mice [45, 46]. Increased Fas ligand has been observed in the mutant SOD1 transgenic mice and spinal cord tissue of ALS patients [47]. In addition, there is evidence for p53 to mediate apoptosis through the Fas-mediated death receptor pathway [4]. Hence it is possible that p53 might act *via* both the caspase driven apoptotic pathway and Fas receptor activation. Fas expression has also been shown to occur in neurons entering the cell cycle to regulate neuronal death during the cell cycle and is tied to cell cycle checkpoints [48]. However, it was intriguing to find Fas receptor in the nucleus of spinal cord motor neurons and co-localized with caspase-8. We performed peptide blocking and other antibody control experiments to demonstrate the specificity of our results. The mode of Fas nuclear translocation or its nuclear function is unknown. However, Fas has been observed in the nucleus of motor neurons after spinal cord injury in an ischemia model system and suggested to participate in DNA damage and apoptosis of motor neurons [49]. Thus from this study we can conclude that a caspase-dependent apoptotic modes of cell death occur in the spinal cord motor neurons but not in neurons of the motor cortex. Although these findings contradict earlier reports wherein alterations of apoptotic proteins were evident both in the spinal cord and motor cortex, our data supports the hypothesis that multiple pathways are involved in motor neuron cell death during ALS.

In conclusion, we report alterations in the G1 to S phase transition of cell cycle with concomitant increases in p53 levels in the spinal cord of ALS patients that may induce an apoptotic mode of spinal motor neuron cell death involving both caspase-3 and Fas. We failed to observe similar alterations of this apoptotic mechanism in the motor cortex. Therefore, these findings suggest differential mechanisms of neuronal cell death in ALS spinal cord and motor cortex.

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ABBREVIATIONS

ALS	=	Amyotrophic lateral sclerosis
EMSA	=	Electrophoretic mobility shift assay
TUNEL	=	Terminal TdT transferase nick-end labeling

REFERENCES

- Dasgupta P, Padmanabhan J, Chellappan S. Rb function in the apoptosis and senescence of non-neuronal and neuronal cells: role in oncogenesis. *Curr Mol Med* 2006; 6: 719-29.
- DeGregori J, Johnson DG. Distinct and overlapping roles for E2F family members in transcription, proliferation and apoptosis. *Curr Mol Med* 2006; 6: 739-48.
- Nahle Z, Polakoff J, Davuluri RV, *et al.* Direct coupling of the cell cycle and cell death machinery by E2F. *Nat Cell Biol* 2002; 4: 859-64.
- Stiewe T, Putzer BM. Role of the p53-homologue p73 in E2F1-induced apoptosis. *Nature Genet* 2000; 26: 464-9.
- Moroni MC, Hickman ES, Denchi EL, *et al.* Apaf-1 is a transcriptional target for E2F and p53. *Nat Cell Biol* 2001; 3: 552-8.
- Luciakova K, Barath P, Li R, Zaid A, Nelson BD. Activity of the human cytochrome c1 promoter is modulated by E2F. *Biochem J* 2000; 351: 251-6.
- Ramalho RM, Ribeiro PS, Sola S, Castro RE, Steer CJ, Rodrigues CM. Inhibition of the E2F-1/p53/Bax pathway by tauroursodeoxycholic acid in amyloid β -peptide-induced apoptosis of PC12 cells. *J Neurochem* 2004; 90: 567-75.
- Blum D, Wu Y, Nissou MF, Arnaud S, Alim Louis B, Verna JM. p53 and Bax activation in 6-hydroxydopamine-induced apoptosis in PC12 cells. *Brain Res* 1997; 751: 139-42.
- Chan SL, Culmsee C, Haughey N, Klapper W, Mattson MP. Presenilin-1 mutations sensitize neurons to DNA damage-induced death by a mechanism involving perturbed calcium homeostasis and activation of calpains and caspase-12. *Neurobiol Dis* 2002; 11: 2-19.
- Daily D, Barzilai A, Offen D, Kamsler A, Melamed E, Ziv I. The involvement of p53 in dopamine-induced apoptosis of cerebellar granule neurons and leukemic cells overexpressing p53. *Cell Mol Neurobiol* 1999; 19: 261-76.
- Inamura N, Araki T, Enokido Y, Nishio C, Aizawa S, Hatanaka H. Role of p53 in DNA strand break-induced apoptosis in organotypic slice culture from the mouse cerebellum. *J Neurosci Res* 2000; 60: 450-7.
- O'Hare MJ, Hou ST, Morris EJ, *et al.* Induction and modulation of cerebellar granule neuron death by E2F-1. *J Biol Chem* 2000; 275: 25358-64.
- Kruman II, Wersto RP, Cardozo-Pelaez F, *et al.* Cell cycle activation linked to neuronal cell death initiated by DNA damage. *Neuron* 2004; 41: 549-61.
- Tieu K, Ashe PC, Zuo DM, Yu PH. Inhibition of 6-hydroxydopamine-induced p53 expression and survival of neuroblastoma cells following interaction with astrocytes. *Neuroscience* 2001; 103: 125-32.
- Jordan J, Galindo MF, Gonzalez-Garcia C, Cena V. Role and regulation of p53 in depolarization-induced neuronal death. *Neuroscience* 2003; 122: 707-15.
- Martin LJ. Neuronal death in amyotrophic lateral sclerosis is apoptosis: Possible contributions of a programmed cell death mechanism. *J Neuropathol Exp Neurol* 1999; 58: 459-71.
- Sathasivam S, Ince PG, Shaw PJ. Apoptosis in amyotrophic lateral sclerosis: A review of the evidence. *Neuropathol Appl Neurobiol* 2001; 27: 257-74.
- Martin LJ. p53 is abnormally elevated and active in the CNS of patients with amyotrophic lateral sclerosis. *Neurobiol Dis* 2000; 7: 613-22.
- Gonzalez de Aguilar J-L, Gordon JW, Rene F, *et al.* Alteration of the Bcl-x/Bax ratio in a transgenic mouse model of amyotrophic lateral sclerosis: evidence for the implication of the p53 signaling pathway. *Neurobiol Dis* 2000; 7: 406-15.
- Bogdanov M, Brown RH, Matson WR, *et al.* Increased oxidative damage to DNA in ALS patients. *Free Radic Biol Med* 2000; 29: 652-8.
- Kikuchi H, Furuta A, Nishioka K, Suzuki SO, Nakabeppu Y, Iwaki T. Impairment of mitochondrial DNA repair enzymes against accumulation of 8-oxo-guanine in the spinal motor neurons of amyotrophic lateral sclerosis. *Acta Neuropathol* 2002; 103: 408-14.
- Shaikh AY, Martin LJ. DNA base-excision repair enzyme apurinic/apyrimidinic endonuclease/redox factor-1 is increased and competent in the brain and spinal cord of individuals with amyotrophic lateral sclerosis. *Neuromol Med* 2002; 2: 47-60.
- Aguirre N, Beal MF, Matson WR, Bogdanov MB. Increased oxidative damage to DNA in an animal model of amyotrophic lateral sclerosis. *Free Radic Res* 2005; 39: 383-8.
- Martin LJ, Chen K, Liu Z. Adult motor neuron apoptosis is mediated by nitric oxide and Fas death receptor linked by DNA damage and p53 activation. *J Neurosci* 2005; 25: 6449-59.
- Eve DJ, Dennis JS, Citron BA. Transcription factor p53 in degenerating spinal cords. *Brain Res* 2007; 1150: 174-81.
- Ranganathan S, Bowser R. Alterations in G1 to S phase cell-cycle regulators during amyotrophic lateral sclerosis. *Am J Pathol* 2003; 162: 823-35.
- Kuntz C, Kinoshita Y, Beal MF, Donehower LA, Morrison RS. Absence of p53: No effect in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Exp Neurol* 2000; 165: 184-90.
- Prudlo J, Koenig J, Graser J, *et al.* Motor neuron cell death in a mouse model of FALS is not mediated by the p53 cell survival regulator. *Brain Res* 2000; 879: 183-7.
- Eliyahu D, Michalovitz D, Eliyahu S, Pinhasi-Kimhi O, Oren M. Wild-type p53 can inhibit oncogene-mediated focus formation. *Proc Natl Acad Sci USA* 1989; 86: 8763-7.
- Finlay CA, Hinds PW, Levine AJ. The p53 proto-oncogene can act as a suppressor of transformation. *Cell* 1989; 57: 1083-93.
- Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* 1991; 352: 345-7.
- Shaw P, Bovey R, Tardy S, Sahli R, Sordat B, Costa J. Induction of apoptosis by wild-type p53 in a human colon tumor-derived cell line. *Proc Natl Acad Sci USA* 1992; 89: 4495-9.
- Donehower LA, Harvey M, Slagle BL, *et al.* Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* 1992; 356: 215-21.
- Harvey M, McArthur MJ, Montgomery CA Jr, Bradley A, Donehower LA. Genetic background alters the spectrum of tumors that develop in p53-deficient mice. *FASEB J* 1993; 7: 938-43.
- Clarke AR, Purdie CA, Harrison DJ, *et al.* Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* 1993; 362: 849-52.
- Ryan KM, Phillips AC, Vousden KH. Regulation and function of the p53 tumor suppressor protein. *Curr Opin Cell Biol* 2001; 13: 332-7.
- Urushitani M, Kurisu J, Tsukita K, Takahashi R. Proteasomal inhibition by misfolded mutant superoxide dismutase I induces selective motor neuron death in FALS. *J Neurochem* 2002; 83: 1030-42.
- Kabashi E, Agar JN, Taylor DM, Minotti S, Durham HD. Focal dysfunction of the proteasome: a pathogenic factor in a mouse model of amyotrophic lateral sclerosis. *J Neurochem* 2004; 89: 1325-35.
- Tsuji S, Kikuchi S, Shinpo K, *et al.* Proteasome inhibition induces selective motor neuron death in organotypic slice cultures. *J Neurosci Res* 2005; 82: 443-51.

- [40] Ou SH, Wu F, Harrich D, Garcia-Martinez LF, Gaynor RB. Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. *J Virol* 1995; 69: 3584-96.
- [41] Davidson Y, Kelley T, Mackenzie IR, *et al.* Ubiquitinated pathological lesions in frontotemporal lobar degeneration contains the TAR DNA-binding protein, TDP-43. *Acta Neuropathol* 2007; 113: 521-33.
- [42] Gilman CP, Chan SL, Guo Z, Zhu X, Greig N, Mattson MP. p53 is present in synapses where it mediates mitochondrial dysfunction and synaptic degeneration in response to DNA damage, and oxidative and excitotoxic insults. *Neuromol Med* 2003; 3: 159-72.
- [43] Pasinelli P, Houseweart MK, Brown RHJ, Cleveland DW. Caspase-1 and -3 are sequentially activated in motor neuron death in Cu,Zn superoxide dismutase-mediated familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* 2000; 97: 13901-6.
- [44] Chi L, Ke Y, Luo C, Gozal D, Liu R. Depletion of reduced glutathione enhances motor neuron degeneration *in vitro* and *in vivo*. *Neuroscience* 2007; 144: 991-1003.
- [45] Raoul C, Estevez AG, Nishimune H, *et al.* Motoneuron death triggered by a specific pathway downstream of Fas. potentiation by ALS-linked SOD1 mutations. *Neuron* 2002; 35: 1067-83.
- [46] Raoul C, Buhler E, Sadeghi C, *et al.* Chronic activation in presymptomatic amyotrophic lateral sclerosis (ALS) mice of a feedback loop involving Fas, Daxx, and FasL. *Proc Natl Acad Sci USA* 2006; 103: 6007-12.
- [47] Kiaei M, Petri S, Kipiani K, *et al.* Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosci* 2006; 26: 2467-73.
- [48] Cheema ZF, Santillano DR, Wade SB, Newman JM, Miranda RC. The extracellular matrix, p53 and estrogen compete to regulate cell-surface Fas/Apo-1 suicide receptor expression in proliferating embryonic cerebral cortical precursors, and reciprocally, Fas-ligand modifies estrogen control of cell-cycle proteins. *BMC Neurosci* 2004; 5: 11.
- [49] Sakurai M, Hayashi T, Abe K, Sadahiro M, Tabayashi K. Delayed selective motor neuron death and Fas antigen induction after spinal cord ischemia in rabbits. *Brain Res* 1998; 797: 23-8.

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