MS-275, a Class I histone deacetylase inhibitor, protects the p53-deficient mouse against ischemic injury

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Abstract

The administration of pan histone deacetylase (HDAC) inhibitors reduces ischemic damage to the CNS, both *in vitro* and in animal models of stroke, via mechanisms which we are beginning to understand. The acetylation of p53 is regulated by Class I HDACs and, because p53 appears to play a role in ischemic pathology, the purpose of this study was to discover, using an *in vitro* white matter ischemia model and an *in vivo* cerebral ischemia model, if neuroprotection mediated by HDAC inhibition depended on p53 expression. Optic nerves were excised from wild-type and p53-deficient mice, and then subjected to oxygen–glucose deprivation in the presence and absence of a specific inhibitor of Class I HDACs (MS-275, entinostat) while compound action potentials were recorded. Furthermore, transient focal ischemia was imposed on wild-type and p53-deficient mice, which were subsequently treated with MS-275. Interestingly, and in both scenarios, the beneficial effects of MS-275 were most pronounced when p53 was absent. These results suggest that modulation of p53 activity is not responsible for MS-275-mediated neuroprotection, and further illustrate how HDAC inhibitors variably influence p53 and associated apoptotic pathways.

Keywords

histone deacetylase; ischemia; mouse optic nerve; MS-275; neuroprotection; p53

Epigenetic mechanisms are integral to biological regulation across the life span and can enhance the intrinsic potential for repair within the CNS (Handel *et al*. 2009; Qureshi and Mehler 2010; Pearce 2011; Jakovcevski and Akbarian 2012; Schweizer *et al*. 2013). Perhaps most striking are the results obtained with histone deacetylase (HDAC) inhibitors (Chuang *et al*. 2009; Haberland *et al*. 2009; Thomas 2009; Fischer *et al*. 2010). For example, the acetylation status of the tumor suppressor p53 is regulated by specific HDACs, and increased acetylation and accumulation promotes growth arrest and apoptosis (Luo *et al*. 2000; Yao and Yang 2011). Hyper-acetylation, via the administration of HDAC inhibitors, has been clinically proven useful in a variety of cancer trials and these drugs are also

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effective in animal models of several inflammatory diseases (Shakespear *et al*. 2011). Recently, Brochier *et al*. (2013) identified the relevant p53 acetylation sites and this has provided a useful molecular understanding of the specific outcomes of HDAC inhibition.

While HDAC inhibitors promote the death of transformed, de-differentiated cells, these same drugs protect differentiated cells, such as neurons, from stressors that would normally lead to apoptosis (Uo *et al*. 2007, 2009). Indeed, the application of pan HDAC inhibitors (Dokmanovic *et al*. 2007) is a proven strategy with which to reduce experimental ischemic injury to the CNS, both *in vitro* (Langley *et al*. 2008; Baltan *et al*. 2011a; Baltan 2012) and in animal models of stroke (Chuang *et al*. 2009; Langley *et al*. 2009; Gibson and Murphy 2010).

As there appears to be a detrimental role for p53 in ischemic pathology (Crumrine *et al*. 1994; Culmsee *et al*. 2001; Leker *et al*. 2004; Endo *et al*. 2006; Vaseva *et al*. 2012), we set out to determine whether HDAC inhibitor-mediated neuroprotection required p53. We administered MS-275, reported to be a specific inhibitor of Class I HDACs (Hess-Stump *et al*. 2007), because Class 1 HDACs are implicated in p53 acetylation and we have shown previously that MS-275 is neuroprotective *ex vivo* (Baltan *et al*. 2011a; Baltan 2012). However, MS-275 has yet to be tested in a stroke model *in vivo*. Interestingly, we find that the neuroprotection elicited by MS-275, both *in vivo* and *ex vivo*, is most clearly apparent when p53 is absent.

Experimental procedures

Materials

The MS-275 was purchased from Selleck (Ontario, London). Sources of antibodies were as follows: cleaved caspase 3 (#9661, 1 : 1000; Cell Signaling Technology, Beverly, MA, USA), histone 3 (#9715, 1:1000; Cell Signaling), acetylated histone 3 (#06–599, final 1 : 5000; Millipore Corporation, Bedford, MA, USA), β-actin (AC-15, 1: 20 000; Sigma, St. Louis, MO, USA), neurofilament 160/200 (N2912, 1:100; Sigma), and donkey anti-mouse Cy3 (1 : 100; Jackson Immuno-Research, West Grove, PA, USA). The mitoCFP mice (Misgeld *et al*. 2007) were originally purchased from Jackson Laboratory and then crossed with p53$^{-/-}$ mice (Donehower *et al*. 1992) at the University of Washington. This study was in compliance with the ARRIVE guidelines.

Transient middle cerebral artery occlusion (MCAo)

This was performed as recently described (Jayadev *et al*. 2011), using male C57bl/6 mice (25–30 g), with approval from the Institutional Animal Care and Use Committee of the University of Washington. Anesthesia was induced by inhalation of 3% isoflurane and maintained by inhalation of 1.5% isoflurane. Body temperature was monitored throughout surgery (via a rectal probe) and maintained at 36–38°C using a heating blanket. Transient focal cerebral ischemia was induced by occlusion of the right middle cerebral artery for 60 min with a filament. Either MS-275 (30 mg/kg) or vehicle (0.05 N HCl and 0.1% Tween in phosphate buffered saline) was given ip immediately upon reperfusion, and again 24 and also 48 h later. Mice were killed 24 h after the final dose of MS-275, and the brain removed

and cut into 1 mm sections using a mouse brain matrix (ASI Instruments, Warren, NJ, USA). To quantitate ischemic damage, brain slices were stained with 2,3,5-triphenyltetrazolium chloride (1 mg/mL saline). Digital photographs were taken and infarct areas calculated by at least two observers, who were blinded to genotype and treatment. All data are presented as mean of infarct (expressed as % hemisphere) ± SEM.

Recording techniques for optic nerve function and oxygen–glucose deprivation

This was performed as previously described (Baltan *et al.* 2011a). Mouse optic nerves (MONs) were gently freed from their dural sheaths and placed in a Haas top chamber superfused with artificial CSF (ACSF) at 37°C. Suction electrodes back-filled with ACSF were used for stimulation and recording the compound action potential (CAP). During experiments, a supramaximal CAP was elicited every 30 s. Oxygen–glucose deprivation (OGD) was induced by switching to glucose-free ACSF (replaced with equimolar sucrose to maintain osmolarity) and a gas mixture containing 95% N_2/5% CO_2. This was applied for 60 min, glucose-containing ACSF and O_2 were restored, and CAP recorded for up to 5–6 h. Optic nerve function was assessed as the area under the supramaximal CAP. Irreversible injury was measured by determining residual CAP area, normalized to control CAP area, 5–6 h after the conclusion of OGD. Data were normalized by setting the mean of initial baseline values (measured over 20 min) to a value of 1.0. Results from several nerves were pooled, averaged, and plotted against time. Data are presented as mean ± SEM.

Immunohistochemistry

This was performed in perfusion-fixed (4% paraformaldehyde in phosphate-buffered saline) MONs. Cryoprotection was achieved in increasing sucrose concentrations (10–30% sucrose). Sections (16 μm) were blocked and permeabilized in 5% normal goat/donkey (50% by volume) serum (Sigma) and 0.3% Triton X-100 (Sigma) for 60 min at 23–25°C. The antibody against neurofilament was used at room temperature for 2 h. After a thorough wash in phosphate-buffered saline, the tissue was exposed to Donkey anti-mouse Cy3 in 2% normal goat serum for 2 h.

Imaging and pixel-intensity measurements

Expression of NF160/200 was imaged using a Leica TCS SP5 upright confocal, whereas cyan fluorescent protein (CFP) was imaged using a Leica TCS SP511 upright multiphoton laser scanning microscope (Buffalo Grove, IL, USA). For NF160/200, sections were scanned with an argon and He–Ne laser for Cy3/543. Two to three adjacent sections from each MON were imaged for a total of five areas of interest. A total of 10–16 optical sections of 1-lm thickness at 512 9 512 pixel size were collected in the *z*-axis from a single microscopic field using 100x (HCX PLAN APO, oil immersion; numerical aperture 1.35; Leica) objective lens under fixed gain, laser power, pinhole, and photomultiplier tube (PMT) settings. For CFP, two to three adjacent sections for each MON were imaged with the wavelength set at 900 nm. A total of 10 optical sections of 1-lm thickness at 512 9 512 pixel size were collected in the z-axis from a single microscopic field using the 25x (HCX IRAPO, water immersion; numerical aperture, 0.95) objective lens under fixed gain, laser power, pinhole, and PMT settings. To compare pixel intensity and quantify immunohistochemical staining, all sections were processed concurrently with Leica imaging

software (LAS-AF version 2.7; Buffalo Grove, IL, USA) using a single channel. The *Z*-stacks were projected into a single plane image before analysis and assessment of pixel intensity.

Cell death assay and western blotting

Immunofluorescence, preparation of protein extracts, and western blotting were carried out as described previously (Uo *et al.* 2005).

Statistical analysis

Data were summarized by means and standard errors. Mean values were compared by Student's *t*-test and *p*-values < 0.05 were considered statistically significant.

Results

The functional integrity of optic nerve axons was monitored by quantifying the area under CAPs evoked by a supramaximal stimulus. After baseline recording, MS-275 was introduced for 30 min and the superfusion conditions were maintained during 60 min of OGD and the initial 30 min of reperfusion. We had reported previously that MS-275 does not affect basic axon function (Baltan *et al.* 2011a). The MON was then reperfused in ACSF for 5 h in the absence of the MS-275.

With the onset of OGD there was a rapid loss of axon function, and this partially recovered when oxygen and glucose were restored. While there was permanent CAP deficit in MONs from both wild-type and p53$^{-/-}$ mice, inclusion of MS-275 in the organ bath not only reduced the extent of CAP loss but also significantly improved recovery in both genotypes (Fig. 1). Interestingly, the effect of MS-275 on CAP recovery in p53$^{-/-}$ MONs was twofold that of MS-275 in wild-type mice (Fig. 2). As these mice express CFP in neuronal mitochondria we were able to image these organelles exclusively in axons of the optic nerve (Fig. 3). The increase in normalized CFP fluorescence intensity in sections taken from MONs functionally protected by MS-275 was elevated more than threefold in p53$^{-/-}$ as compared with wild type, implying enhanced preservation of mitochondrial viability. The neuroprotective effect of MS-275 was confirmed by assessing the expression of NF160/200 in adjacent sections (Fig. 4).

To validate the central effects of MS-275, we first delivered 30 mg/kg ip to mice after stroke, solubilized brain, and ran western blots for acetylated histone 3, total H3, and actin. It is clear that MS-275 accessed the brain to cause HDAC inhibition, as acetylated histone 3 was more abundant in these brains compared to those of untreated mice (Fig. 5). Then we looked at the effects of MS-275 and p53 expression on anatomic outcome after MCAo. Three days after occlusion of the right MCA for 60 min, there was an infarct that involved approximately 50% of the right hemisphere in wild-type mice (Fig. 6). As previously observed, mice devoid of p53 generally had smaller infarcts, though this failed to reach significance. While the administration of MS-275 post-stroke did not affect infarct size in wild-type mice, the p53$^{-/-}$ mice receiving MS-275 had consistently smaller infarcts.

Finally, as other HDAC inhibitors show protection against MCAo in wild-type mice (Gibson and Murphy 2010), we looked at the effects of MS-275 on p53 expression in neurons in culture (Fig. 7). In previous studies, addition of suberoylanilide hydroxamate and TSA were shown to dramatically increase p53 expression at the same time as suppressing p53-mediated transcriptional activity and cell death (Uo *et al.* 2009). As expected, both camptothecin and nutlin exposure increased p53 expression but, in contrast to other HDAC inhibitors, MS-275 suppressed p53 expression.

Discussion

Our previous results from *ex vivo* (optic nerve) and *in vitro* (cultured neuron) studies support the notion that HDAC inhibitors engage a diverse array of targets involved in the maintenance of cell function and cell survival in the face of injury (Haberland *et al.* 2009). Potential explanations for the beneficial effects of compounds such as MS-275 are numerous (Baltan *et al.* 2013). For example, HDAC inhibitors can promote neuronal survival by blocking the action of p53 (Uo *et al.* 2009; Brochier *et al.* 2013), which has been implicated in acute CNS injury (Morrison *et al.* 2003). The apoptotic activity of p53 in post-natal cortical neurons is exclusively mediated through transcription-dependent functions (Uo *et al.* 2007) and HDAC inhibitors protect cultured post-natal cortical neurons from p53-dependent cell death by suppressing PUMA expression, a critical signaling intermediate linking p53 to Bax activation (Uo *et al.* 2009). Although many HDAC inhibitors (such as suberoylanilide hydroxamate, TSA) cause p53 to accumulate in neurons, despite blocking p53 transcriptional activity, we observed that MS-275 treatment reduced p53 accumulation after DNA damage and this may reflect a different mechanism of action (Uo *et al.* 2009). It is conceivable that MS-275, in contrast to other HDAC inhibitors, does not directly affect p53 acetylation, or its stability and activity. This drug might indirectly reduce p53 levels, without causing a significant decline in p53 activity, and this could explain why MS-275 is hardly effective in the presence of p53.

If the target for MS-275 is not p53, the drug could modulate neuronal survival by modifying the activity of other transcription factors. For example, nuclear erythroid 2-related factor 2 promotes multiple pathways of cellular protection by coordinating the transcription of genes that confer protection against oxidative stress and inflammation (Martín-Montalvo *et al.* 2011; Wang *et al.* 2012). Activation of the nuclear erythroid 2-related factor 2 pathway has been shown to increase the resistance of neurons to oxidative stress and metabolic insults *in vitro*, and to ischemic stroke *in vivo* (Son *et al.* 2010).

During ischemic injury, and consistent with increased fission and the loss of ATP, morphological changes in mitochondria are induced in the mouse optic nerve. However, HDAC inhibitors like MS-275 preserve mitochondria and maintain ATP (current studies; Baltan *et al.* 2011a). We have also found that a period of OGD depletes the glutamate transporter 1 transporter in optic nerve, but that the administration of HDAC inhibitors robustly up-regulates expression of this pump and prominently delays and attenuates glutamate release (Baltan *et al.* 2011a).

Which HDACs are implicated in stroke pathology? Based on the outcome of shRNA knockdown in cultured primary neurons (RS Morrison, unpublished observation), our focus has been on those of Class 1. Recently, we mapped the cell- and region-specific expression of HDACs 1, 2, and 3 in mouse brain, and reported that a period of ischemia up-regulates expression of these enzymes (Baltan *et al.* 2011b). Deletion of each of the Class 1 HDACs is embryonically lethal, mainly through effects on cardiac development, and for HDAC 3 the issue is defective repair of DNA double-stranded breaks (Haberland *et al.* 2009). There appear to be distinct roles for Class 1 HDACs in neuronal differentiation. The activity of HDAC 1 is required for differentiation to neurons, whereas HDAC 2 blocks differentiation to oligodendrocytes and inhibits differentiation to astrocytes (Humphrey *et al.* 2008). Both HDACs 1 and 2 are also involved in precursor cell maturation (Montgomery *et al.* 2009). Although it is not at all clear if the notable amplification of neurogenesis post-stroke contributes to subsequent restoration of function, the ability of HDAC inhibitors to promote migration and 'neuronal' differentiation could be exploited, therapeutically (Kahle and Bix 2013).

Although MS-275 selectively inhibits Class 1 HDACs (Hess-Stump *et al.* 2007; Khan *et al.* 2008), this drug does not discriminate between HDACs 1, 2, and 3 (Lauffer *et al.* 2013), and there is good evidence to suggest that inhibition of HDAC 1 in the CNS could be detrimental (Kim *et al.* 2008). Activity of HDAC 2 counteracts memory formation and over-expression impairs synaptic plasticity (Guan *et al.* 2009). HDAC 2 modulates nuclear factor kappa B and contributes to the signaling linked to cell proliferation, survival and differentiation, and pharmacological intervention to precisely target HDAC 2 is considered to be a valid therapeutic option (Kramer 2009). Recently, the neurotoxicity associated with mutant Huntingtin was shown to be much reduced in the absence of HDAC 3 (Bardai *et al.* 2013). The search for more selective inhibitors of HDACs 2 and 3 continues (Thomas 2009; Fischer *et al.* 2010; Durham 2012).

In summary, the Class 1 HDAC inhibitor, MS-275, ameliorates ischemic pathology in the CNS more robustly in the absence of p53, distinguishing its activity from other HDAC inhibitors. The acute (preserving white matter) and longer term effects of HDAC inhibitors (reducing cell death, promoting progenitor cell differentiation) suggest these drugs to be potential stroke therapeutics. Differences between broadly effective HDAC inhibitors, as shown in this study, further underscore the need to develop more selective inhibitors.

Acknowledgements

Abbreviations used:

AcH3	acetylated histone 3
ACSF	artificial CSF

CAP	compound action potential
CFP	cyan fluorescent protein
CT	camptothecin
GFAP	glial fibrillary acidic protein
HDAC	histone deactylase
MCAo	middle cerebral artery occlusion
MON	mouse optic nerve
MS-275	*N*-(2-aminophenyl)-4-[*N*-(pyridine-3yl-methoxy-carbonyl) aminomethyl]benzamide
NF160/200	neurofilament
OGD	oxygen–glucose deprivation
SAHA	suberoylanilide hydroxamate
TTC	2,3,5-triphenyltetrazolium chloride
WM	white matter

References

Baltan S (2012) Histone deacetylase inhibitors preserve function in aging axons. J. Neurochem 12(Suppl. 2), 108–115.

Baltan S, Murphy SP, Danilov CA, Bachleda A and Morrison RS (2011a) Histone deacetylase inhibitors preserve white matter structure and function during ischemia by conserving ATP and reducing excitotoxicity. J. Neurosci 31, 3990–3999. [PubMed: 21411642]

Baltan S, Bachleda A, Morrison RS and Murphy SP (2011b) Expression of histone deacetylases in cellular compartments of the mouse brain and the effects of ischemia. Transl. Stroke Res 2, 411–423. [PubMed: 21966324]

Baltan S, Morrison RS and Murphy SP (2013) Novel protective effects of histone deacetylase inhibition on stroke and white matter ischemic injury. Neurotherapeutics 10, 798–807. [PubMed: 23881453]

Bardai FH, Verma P, Smith C, Rawat V, Wang L and D'Mello SR (2013) Disassociation of HDAC 3 from normal Huntingtin underlies mutant Huntingtin neurotoxicity. J. Neurosci 33, 11833–11838. [PubMed: 23864673]

Brochier C, Dennis G, Rivieccio MA et al. (2013) Specific acetylation of p53 by HDAC inhibition prevents DNA damage-induced apoptosis in neurons. J. Neurosci 33, 8621–8623. [PubMed: 23678107]

Chuang D-W, Leng Y, Marinova Z, Kim H-J and Chiu C-T (2009) Multiple roles of HDAC inhibition in neurodegenerative conditions. Trends Neurosci. 32, 591–601. [PubMed: 19775759]

Crumrine RC, Thomas AL and Morgan PF (1994) Attenuation of p53 expression protects against focal ischemic damage in transgenic mice. J. Cereb. Blood Flow Metab 14, 887–891. [PubMed: 7929653]

Culmsee C, Zhu X, Yu QS et al. (2001) A synthetic inhibitor of p53 protects neurons against death induced by ischemic and excitotoxic insults, and amyloid beta-peptide. J. Neurochem 77, 220–228. [PubMed: 11279278]

Dokmanovic M, Clarke C and Marks PA (2007) Histone deacetylase inhibitors: overview and perspectives. Mol. Cancer Res 5, 981–989. [PubMed: 17951399]

Donehower LA, Harvey M, Slagle BL et al. (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356, 215–221. [PubMed: 1552940]

Durham B (2012) Novel HDAC inhbiitors with improved selectivity for HDAC 2 and 3 protect against cell death. Biosci. Horiz 5, 1–7.

Endo H, Kamada H, Nito C, Nishi T and Chan PH (2006) Mitochondrial translocation of p53 mediates release of cytochrome c and hippocampal CA1 neuronal death after transient global cerebral ischemia in rats. J. Neurosci 26, 7974–7983. [PubMed: 16870742]

Fischer A, Sananbenesi F, Mungenast A and Tsai L-H (2010) Targeting the correct HDAC(s) to treat cognitive disorders. Trends Pharmacol. Sci 31, 605–617. [PubMed: 20980063]

Gibson C and Murphy SP (2010) Benefits of histone deacetylase inhibitors for acute brain injury; a systematic review of animal studies. J. Neurochem 115, 806–813. [PubMed: 20831615]

Guan J-S, Haggarty SJ, Giacometti E et al. (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459, 55–60. [PubMed: 19424149]

Haberland M, Montgomery RL and Olson EN (2009) The many roles for HDACs in development and physiology. Nat. Rev. Gen 10, 32–42.

Handel AE, Ebers GC and Ramagoplan SV (2009) Epigenetics: molecular mechanisms and implications for disease. Trends Mol. Med 16, 7–16. [PubMed: 20022812]

Hess-Stump H, Bracker TU, Henderson D and Politz O (2007) MS-275, a potent orally available inhibitor of HDACs. Int. J. Biochem. Cell Biol 39, 1388–1405. [PubMed: 17383217]

Humphrey GW, Wang Y-H, Hirai T et al. (2008) Complementary roles for HDACs 1, 2, and 3 in differentiation of pluripotent stem cells. Differentiation 76, 348–356. [PubMed: 18021260]

Jakovcevski M and Akbarian S (2012) Epigenetic mechanisms in neurological disease. Nat. Med 18, 1194–1204. [PubMed: 22869198]

Jayadev S, Nesser NK, Hopkins S et al. (2011) The transcription factor p53 influences microglia activation phenotype. Glia 59, 1402–1413. [PubMed: 21598312]

Kahle MP and Bix GJ (2013) Neuronal restoration following ischemic stroke: influences, barriers, and therapeutic potential. Neurorehabil. Neural Repair 27, 469–478. [PubMed: 23392917]

Khan N, Jeffers M, Kumar S et al. (2008) Determination of the class and isoform selectivity of small-molecule histone deacetylase inhibitors. Biochem. J 409, 581–589. [PubMed: 17868033]

Kim D, Frank CL, Dobbin MM et al. (2008) Deregulation of HDAC1 by p25/Cdk5 in neurotoxicity. Neuron 60, 803–817. [PubMed: 19081376]

Kramer OH (2009) HDAC2: a critical factor in health and disease. Trends Pharmacol. Sci 30, 647–655. [PubMed: 19892411]

Langley B, D'Annibale MA, Suh K et al. (2008) Pulse inhibition of HDACs induces complete resistance to oxidative death in cortical neurons without toxicity and reveals a role for cytoplasmic p21 waf1/cip1 in cell cycle-independent neuroprotection. J. Neurosci 28, 163–176. [PubMed: 18171934]

Langley B, Brochier C and Rivieccio MA (2009) Targeting HDACs as a multifaceted approach to treat the diverse outcomes of stroke. Stroke 40, 2899–2905. [PubMed: 19478231]

Lauffer BEL, Mintzer R, Fong R et al. (2013) Histone deacetylase (HDAC) inhibitor kinetic rate constants correlate with cellular histone acetylation but not transcription and cell viability. J. Biol. Chem 288, 26926–26943. [PubMed: 23897821]

Leker RR, Aharonowiz M, Greig NH and Ovadia H (2004) The role of p53-induced apoptosis in cerebral ischemia: effects of the p53 inhibitor pifithrin alpha. Exp. Neurol 187, 478–486. [PubMed: 15144874]

Luo J, Su F, Chen D, Shiloh A and Gu W (2000) Deacetylation of p53 modulates its effect on cell growth and apoptosis. Nature 408, 377–381. [PubMed: 11099047]

Martín-Montalvo A, Villalba JM, Navas P and de Cabo R (2011) NRF2, cancer and calorie restriction. Oncogene 30, 505–520. [PubMed: 21057541]

Misgeld T, Kerschensteiner M, Bareyre FM, Burgess RW and Lichtman JW (2007) Imaging axonal transport of mitochondria in vivo. Nat. Methods 4, 559–561. [PubMed: 17558414]

Montgomery RL, Hsieh J, Barbosa AC, Richardson JA and Olson EN (2009) HDACs 1 and 2 control the progression of neural precursors to neurons during brain development. Proc. Natl Acad. Sci. USA 106, 7876–7881. [PubMed: 19380719]

Morrison R, Kinoshita Y, Johnson MD, Guo W and Garden GA (2003) p53-dependent cell death signaling in neurons. Neurochem. Res 28, 15–27. [PubMed: 12587660]

Pearce WJ (2011) Epigenetics: an expanding new piece of the stroke puzzle. Transl. Stroke Res 2, 243–247. [PubMed: 21987632]

Qureshi IA and Mehler MF (2010) Emerging role of epigenetics in stroke. Part 1: DNA methylation and chromatin modifications. Arch. Neurol 67, 1316–1322. [PubMed: 21060009]

Schweizer S, Meisel A and Marschenz S (2013) Epigenetic mechanisms in cerebral ischemia. J. Cereb. Blood Flow Metab 33, 1335–1346. [PubMed: 23756691]

Shakespear MR, Halili MA, Irvine KM, Fairlie DP and Sweet MJ (2011) Histone deacetylases as regulators of inflammation and immunity. Trends Immunol. 32, 335–343. [PubMed: 21570914]

Son TG, Camandola S, Arumugam TV et al. (2010) Plumbagin, a novel Nrf2/ARE activator, protects against cerebral ischemia. J. Neurochem 112, 1316–1326. [PubMed: 20028456]

Thomas EA (2009) Focal nature of neurological disorders necessitates isotype-selective HDAC inhibitors. Mol. Neurobiol 40, 33–45. [PubMed: 19396637]

Uo T, Kinoshita Y and Morrison RS (2005) Neurons exclusively express N-Bak, a BH3 domain-only Bak isoform that promotes neuronal apoptosis. J. Biol. Chem 280, 9065–9073. [PubMed: 15590665]

Uo T, Kinoshita Y and Morrison RS (2007) Apoptotic actions of p53 require transcriptional activation of PUMA and do not involve a direct mitochondrial/cytoplasmic site of action in postnatal cortical neurons. J. Neurosci 27, 12198–12210. [PubMed: 17989286]

Uo T, Veenstra TD and Morrison RS (2009) HDAC inhibitors prevent p53-dependent and -independent Bax-mediate neuronal apoptosis through two distinct mechanisms. J. Neurosci 29, 2824–2832. [PubMed: 19261878]

Vaseva AV, Marchenko ND, Ji K, Tsirka SE, Holzmann S and Moll UM (2012) p53 opens the mitochondrial permeability transition pore to trigger necrosis. Cell 149, 1536–1548. [PubMed: 22726440]

Wang B, Zhu X, Kim YT et al. (2012) HDAC inhibition activates transcription factor Nrf2 and protects against cerebral ischemic damage. Free Radic. Biol. Med 52, 928–936. [PubMed: 22226832]

Yao Y-L and Yang W-M (2011) Beyond histone and deacetylase: an overview of cytoplasmic HDACs and their non-histone substrates. J. Biomed. Biotech 2011, 146493.
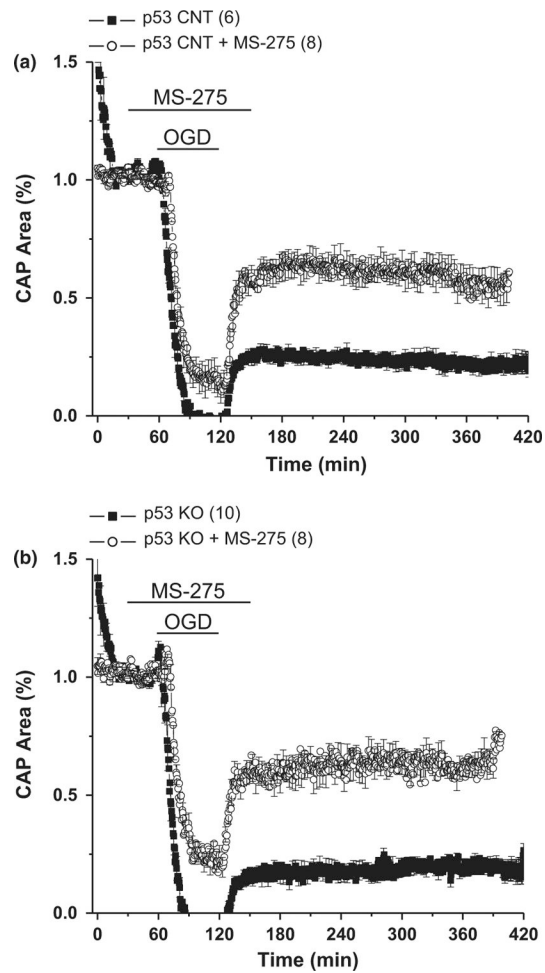
Fig. 1.

The onset of a period of ischemia [oxygen–glucose deprivation (OGD)] in mouse optic nerve caused immediate loss of axon function and incomplete recovery (filled squares) in both (a) wild-type (WT) and (b) p53 knockout (KO) mice. Addition of the histone deacetylase (HDAC) inhibitor MS-275 (1 μM) prior to oxygen–glucose deprivation (OGD) (open circles) somewhat ameliorated loss of function but dramatically improved compound action potential (CAP) recovery in both genotypes. Number of optic nerves in parentheses.
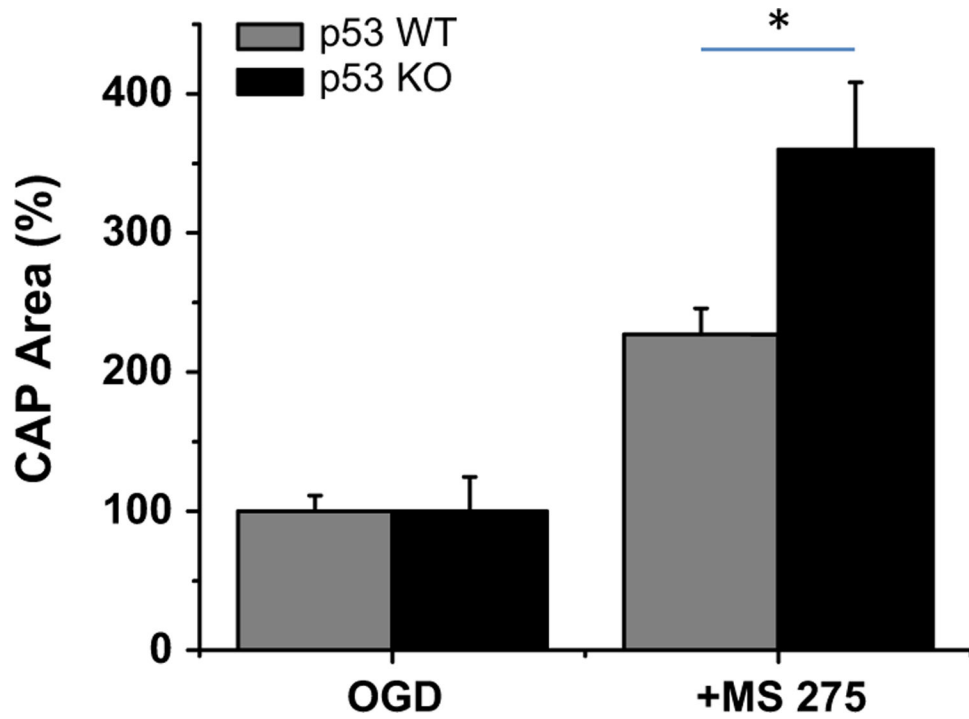
Fig. 2.
Recovery of compound action potential (CAP) area after oxygen–glucose deprivation (OGD) in the presence of MS-275 was significantly greater in optic nerves from p53$^{-/-}$ as compared with wild-type mice. $*p < 0.02$.
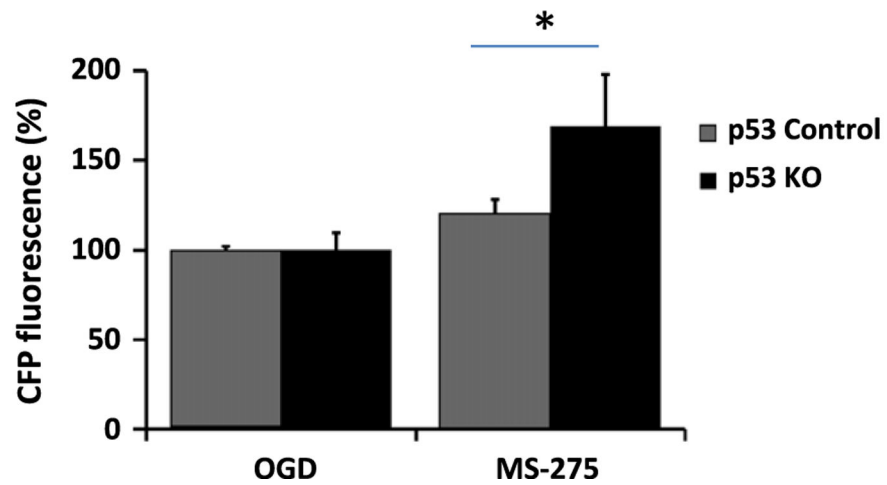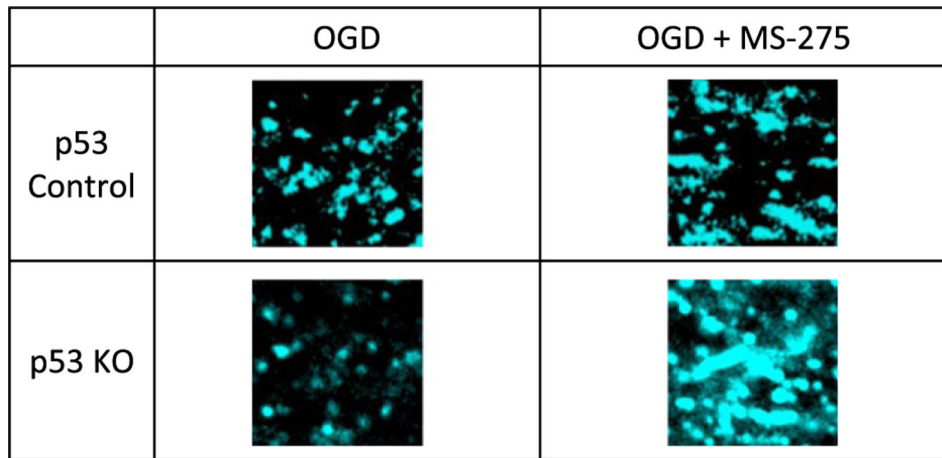
Fig. 3.
Images of neuronal [cyan fluorescent protein (CFP+)] mitochondria in mouse optic nerve (MON) from wt and p53 ko mice after oxygen–glucose deprivation (OGD), and in the absence or presence of MS-275. The histogram summarizes data from a minimum of three mice (± SEM) for each group. *$p < 0.05$.
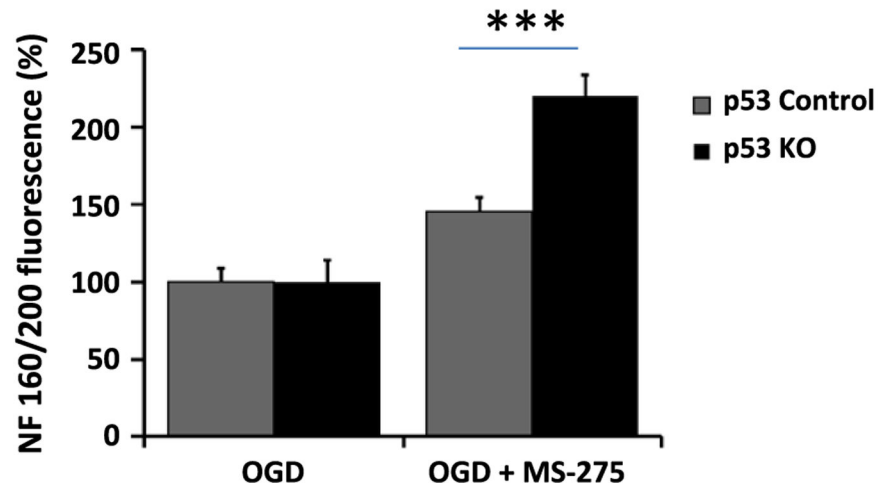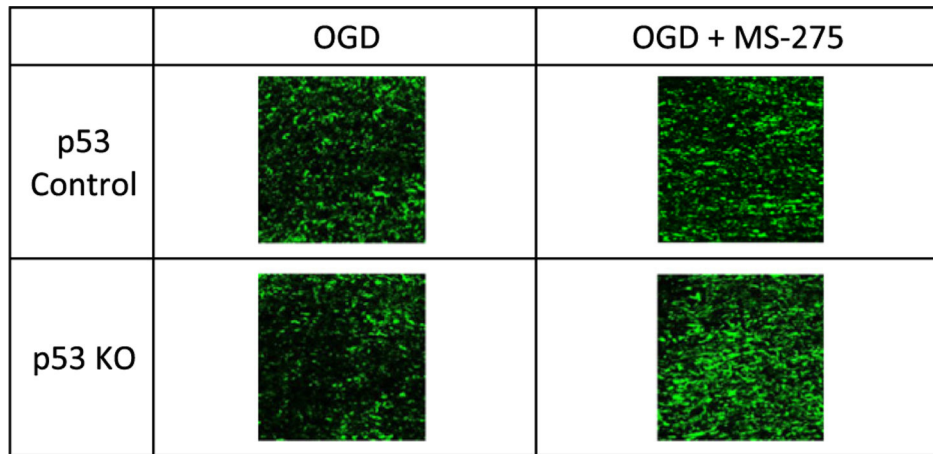
Fig. 4.
Images of NF160/200 in axons of mouse optic nerve (MON) from wt and p53 ko mice after oxygen–glucose deprivation (OGD), and in the absence or presence of MS-275. The histogram summarizes data from a minimum of three mice (± SEM) for each group. ***$p <$ 0.01.
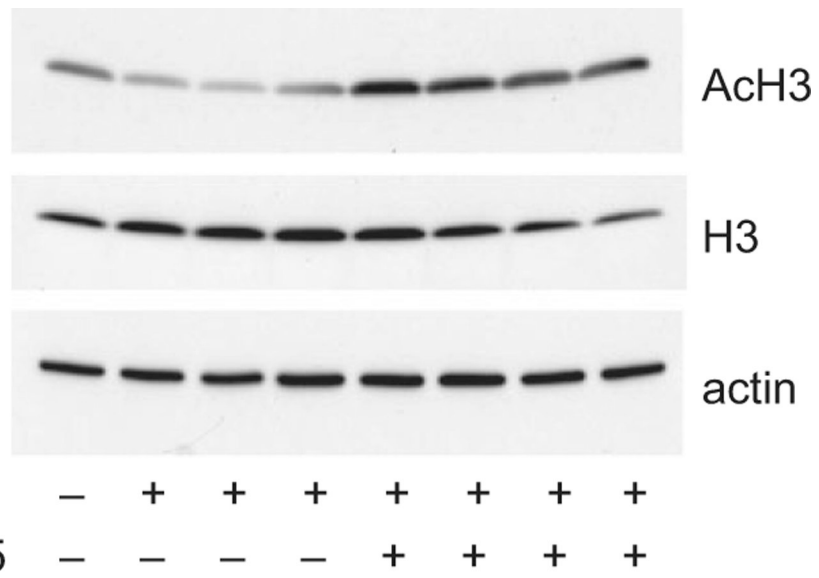
Fig. 5.
Intraperitoneal injection of MS-275 after middle cerebral artery occlusion (MCAo) resulted
in the accumulation of acetylated histone protein 3 (AcH3) in mouse brain, as revealed
by western blotting. Each lane comprises protein extracted from a different animal. Total
histone protein 3 (H3) and β-actin served as controls.
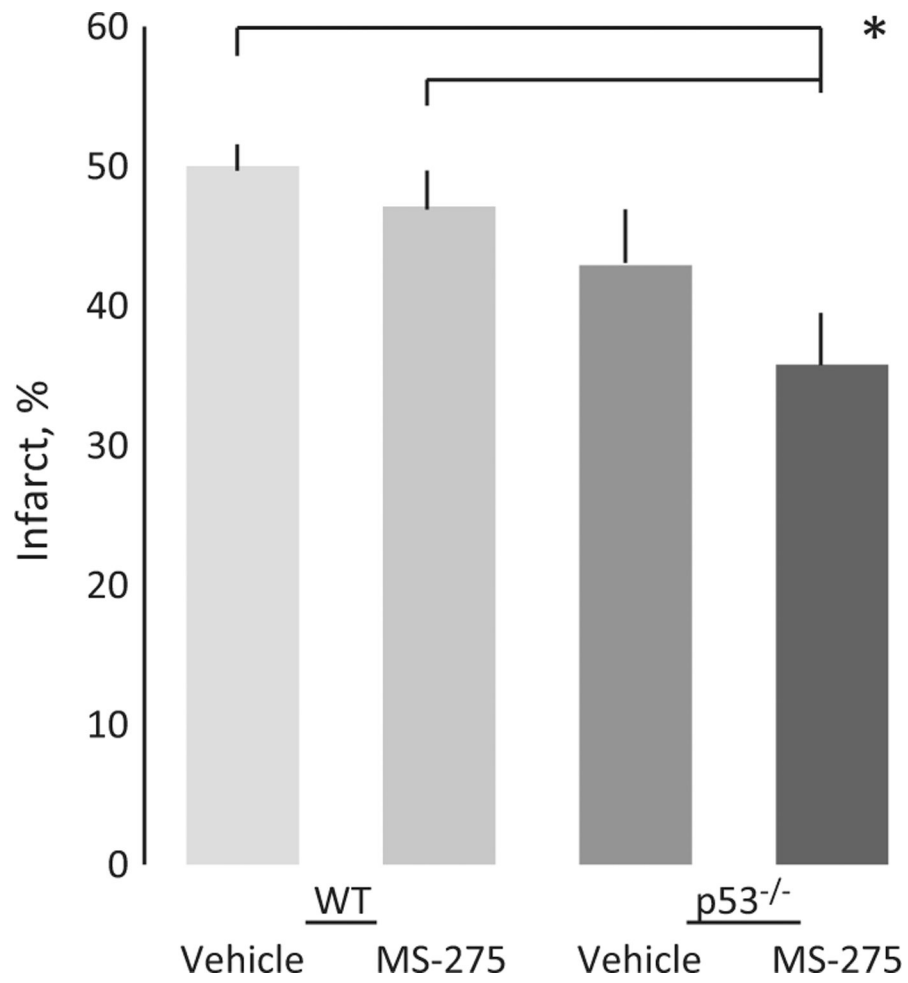
Fig. 6.
The ip administration of MS-275 after 60 min middle cerebral artery occlusion (MCAo) resulted in smaller infarcts (% hemisphere) in p53$^{-/-}$ as compared with wild-type mice. $n = 10 \pm$ SEM for each group. *$p < 0.05$.
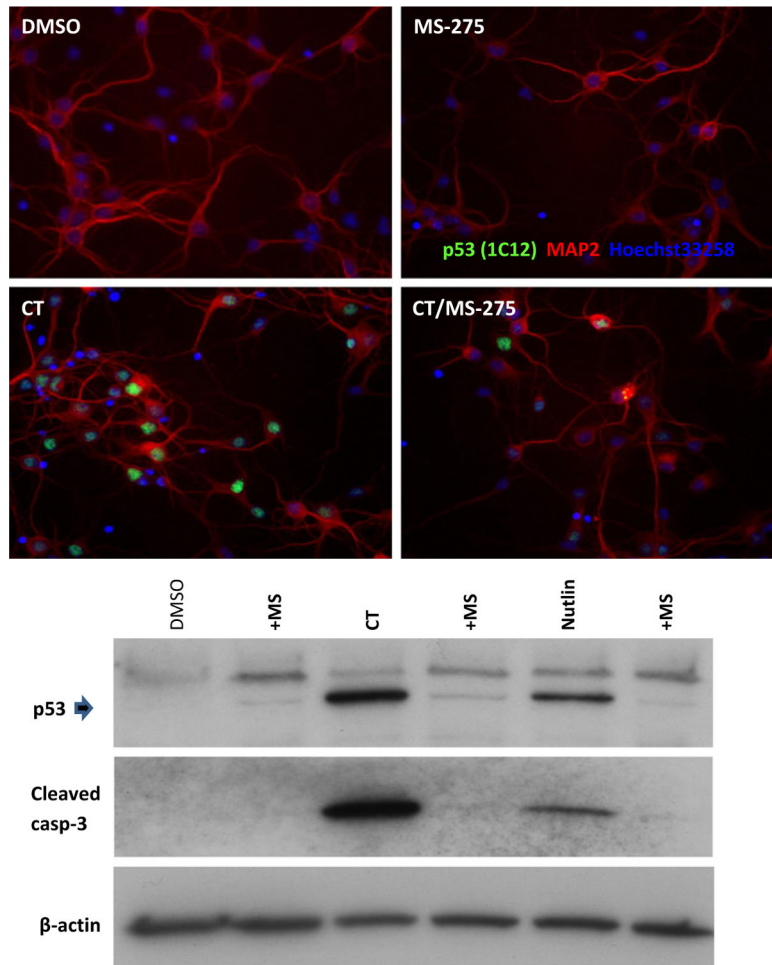
Fig. 7.
Administration of MS-275 to cortical neurons in culture prevented the accumulation of p53 and cleaved caspase 3 invoked by exposure to camptothecin (CT) and nutlin. Neurons were treated with CT (2.5 μM) in the presence or absence of MS-275 (10 μM) for 12 h (DMSO as a vehicle control), fixed, and stained for p53 and a neuronal marker, MAP2, with Hoechst 33258 staining of nuclei. Protein samples were prepared and subjected to western blotting for p53 and cleaved caspase 3 expression. β-actin was the control for sample loading.