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Radiation-induced astrocyte senescence is rescued by Δ133p53

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Abstract

Background. Cellular senescence and the senescence-associated secretory phenotype (SASP) may contribute to the development of radiation therapy–associated side effects in the lung and blood vessels by promoting chronic inflammation. In the brain, inflammation contributes to the development of neurologic disease, including Alzheimer's disease. In this study, we investigated the roles of cellular senescence and Δ133p53, an inhibitory isoform of p53, in radiation-induced brain injury.

Methods. Senescent cell types in irradiated human brain were identified with immunohistochemical labeling of senescence-associated proteins $p16^{INK4A}$ and heterochromatin protein Hp1γ in 13 patient cases, including 7 irradiated samples. To investigate the impact of radiation on astrocytes specifically, primary human astrocytes were irradiated and examined for expression of Δ133p53 and induction of SASP. Lentiviral expression of Δ133p53 was performed to investigate its role in regulating radiation-induced cellular senescence and astrocyte-mediated neuroinflammation.

Results. Astrocytes expressing $p16^{INK4A}$ and Hp1γ were identified in all irradiated tissues, were increased in number in irradiated compared with untreated cancer patient tissues, and had higher labeling intensity in irradiated tissues compared with age-matched controls. Human astrocytes irradiated in vitro also experience induction of cellular senescence, have diminished Δ133p53, and adopt a neurotoxic phenotype as demonstrated by increased senescence-associated beta-galactosidase activity, $p16^{INK4A}$, and interleukin (IL)-6. In human astrocytes, Δ133p53 inhibits radiation-induced senescence, promotes DNA double-strand break repair, and prevents astrocyte-mediated neuroinflammation and neurotoxicity.

Conclusions. Restoring expression of the endogenous p53 isoform, Δ133p53, protects astrocytes from radiation-induced senescence, promotes DNA repair, and inhibits astrocyte-mediated neuroinflammation.

Key Points

1. Astrocyte senescence is increased in irradiated human brain tissue.
2. Radiation-induced astrocyte senescence induces neurotoxicity.
3. Δ133p53 inhibits radiation-induced astrocyte senescence to promote neuroprotection.

Importance of the Study

With improvements in cancer therapies, an increasing number of patients survive long enough to experience late complications of radiotherapy, including progressive cognitive impairment that can escalate to severe memory loss and dementia. Astrocytes are ubiquitous brain cells that may promote neuroinflammation and neurotoxicity in neurodegenerative diseases through the adoption of SASP. This study identifies senescent astrocytes in irradiated patient brain tissues and demonstrates that Δ133p53 inhibits radiation-induced astrocyte senescence, promotes DNA repair, and prevents production of neurotoxic IL-6 from irradiated primary human astrocytes.

Cranial radiation therapy is used to effectively treat brain cancer in adult and pediatric patients.[1,2] Since its development, protocols have evolved to incorporate methods to reduce side effects, such as shielding the hippocampus and fractioning the total radiation dose.[3–5] However, even with improvements, over 40% of patients surviving >6 months experience late side effects. In up to 5% of these patients, neurocognitive impairment progresses from decreased attention and problem-solving ability to memory loss, ataxia, and dementia.[6,7] Late effects may also develop in pediatric patients for whom radiation may be prescribed to treat the two most common cancer types: leukemia and glioma.[8–10] Side effects in these patients include deficits in social functioning and vocational difficulty and poor performance in intelligence quotient testing and are most severe in the youngest patients receiving the highest radiation doses.[4,9–13] As the number of cancer survivors increases, it becomes increasingly critical to understand the causes of these late effects and to develop strategies to prevent them.

Side effects of cancer therapy may be associated with injury to non-tumor cells.[14] Following radiation exposure and accumulation of DNA damage, cells may adopt one of several cell type–specific responses, including induction of cellular senescence.[5,15] Importantly, although senescent cells do not replicate, they may avoid clearance and persist in tissues while continuing to produce inflammatory factors that contribute to tissue injury.[16,17] In this way, radiation-induced cellular senescence is being recognized as an important mediator of tissue dysfunction promoting chronic inflammation and contributing to radiation-induced side effects, including pulmonary fibrosis and cerebrovascular dysfunction.[18,19]

To investigate the role of cellular senescence in cranial radiotherapy, this study examines brain tissue from patients who have undergone brain radiation treatment and identifies several senescent cell types, including astrocytes. Astrocytes perform many neuroprotective functions, including production of neurotrophic factors. However, astrocytes may also promote neurodegeneration in some diseases, including Alzheimer's disease, which is thought to be related to induction of a senescence-associated secretory phenotype (SASP).[17,20] The role of astrocytes and astrocyte senescence in radiation-induced brain injury has not been previously characterized.[6]

After identifying senescent astrocytes in irradiated tissues, this study investigates the potential functions of astrocyte senescence and SASP in promoting brain injury. Based on previous studies[20] identifying regulation of replicative senescence by one of the p53 isoforms, Δ133p53, this study examines the role of Δ133p53 in regulating radiation-induced astrocyte senescence. These findings identify restoration of Δ133p53 as a potential therapeutic approach to inhibiting radiation-induced astrocyte senescence, promoting DNA repair in irradiated astrocytes, and preventing astrocyte-mediated neuroinflammation.

Methods

Human Patient Tissues

Case tissues were acquired with full institutional review board approval from the Georgetown Brain Bank, the Histopathology Tissue Shared Resource at Georgetown University, and Johns Hopkins Brain Bank and included non-tumor brain tissue from cancer patients with a history of cranial radiation treatment, with no history of treatment, or from non-disease, age-matched controls collected at autopsy (Supplementary Table 1). Patients receiving chemotherapy or immunotherapy were excluded.[14] Tissues were anonymized, labeled with senescence-associated proteins (Supplementary Table 2), and examined by 3 pathologists (J.B., B.H., I.O.). Each control and radiation-treated tissue was assigned an immunoreactivity score in a blinded manner based on the intensity of immunohistochemical labeling

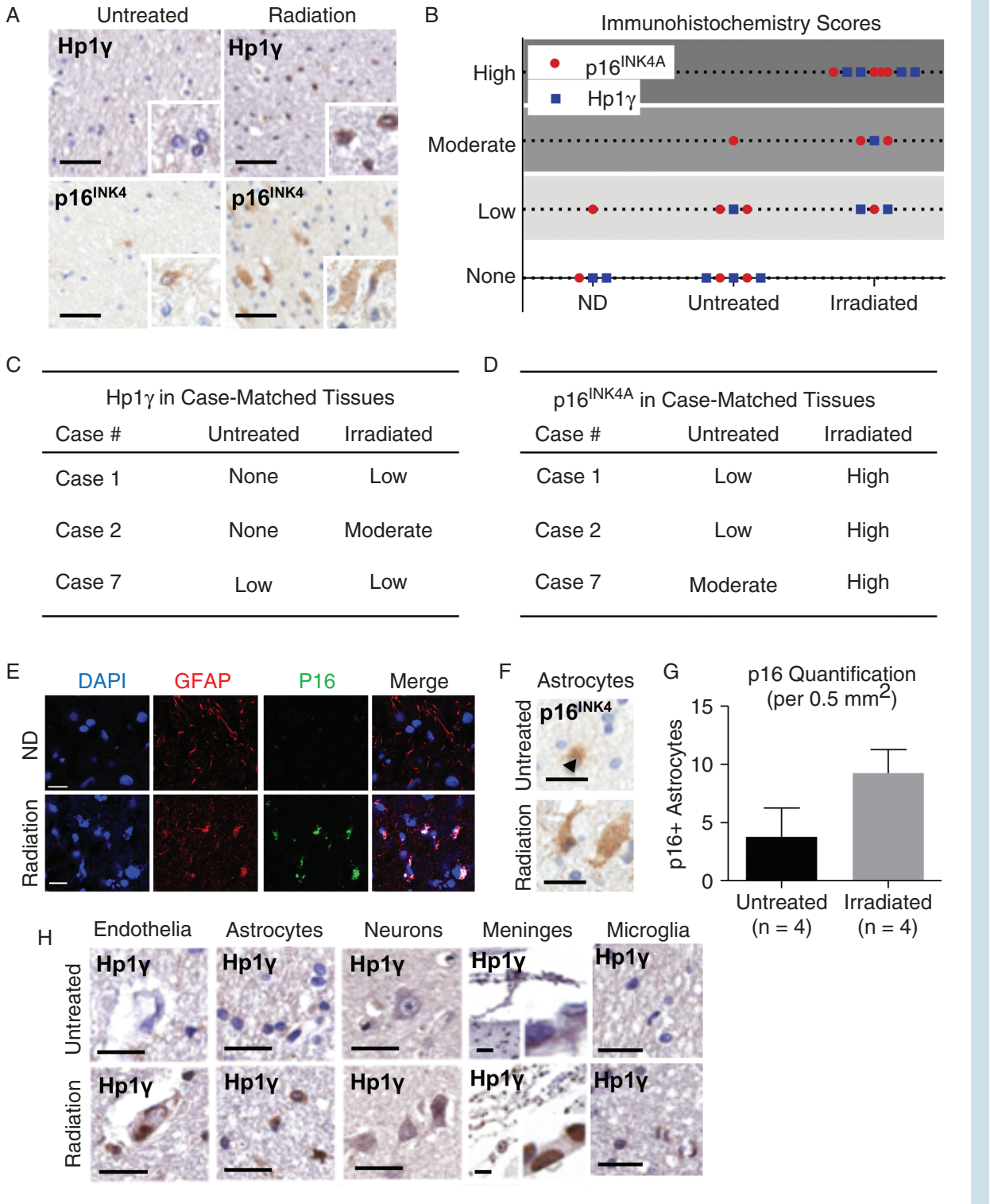

Fig. 1 Astrocyte senescence is increased in irradiated patient tissues. (A) Expression of senescence-associated proteins Hp1γ and $p16^{INK4}$ in irradiated and untreated non-tumor brain tissues using immunohistochemistry. (B) Tissues were examined in a blinded fashion by 3 pathologists and scored from 0 (none) to 3 (high) based on intensity of cell labeling. (C) Hp1γ and (D) $p16^{INK4A}$ immunohistochemical labeling in 3 patients receiving stereotactic radiation with comparison of irradiated to untreated tissue in the same patient as an internal control. *Case 7 is from a patient with a previous diagnosis of Alzheimer's disease in which astrocyte senescence is prominent and thought to promote neurodegeneration.[20] (E) Immunocytochemistry of irradiated brain tissue demonstrating co-localization of $p16^{INK4A}$ and GFAP in astrocytes. (F) $p16^{INK4A}$-positive astrocytes in irradiated human brain tissues using immunohistochemistry. (G) Quantification of $p16^{INK4A}$-positive astrocytes in 20 microscopic fields (0.5 mm^2) in non-tumor brain tissue from untreated cancer patients ($n = 4$) and cancer patients receiving radiation treatment ($n = 4$). (H) Representative images of cell types expressing senescence-associated Hp1γ in irradiated (stereotactic) and untreated brain tissue from the same patient, including endothelia, astrocytes, neurons, meninges, and microglia. Scale = 50 μm.

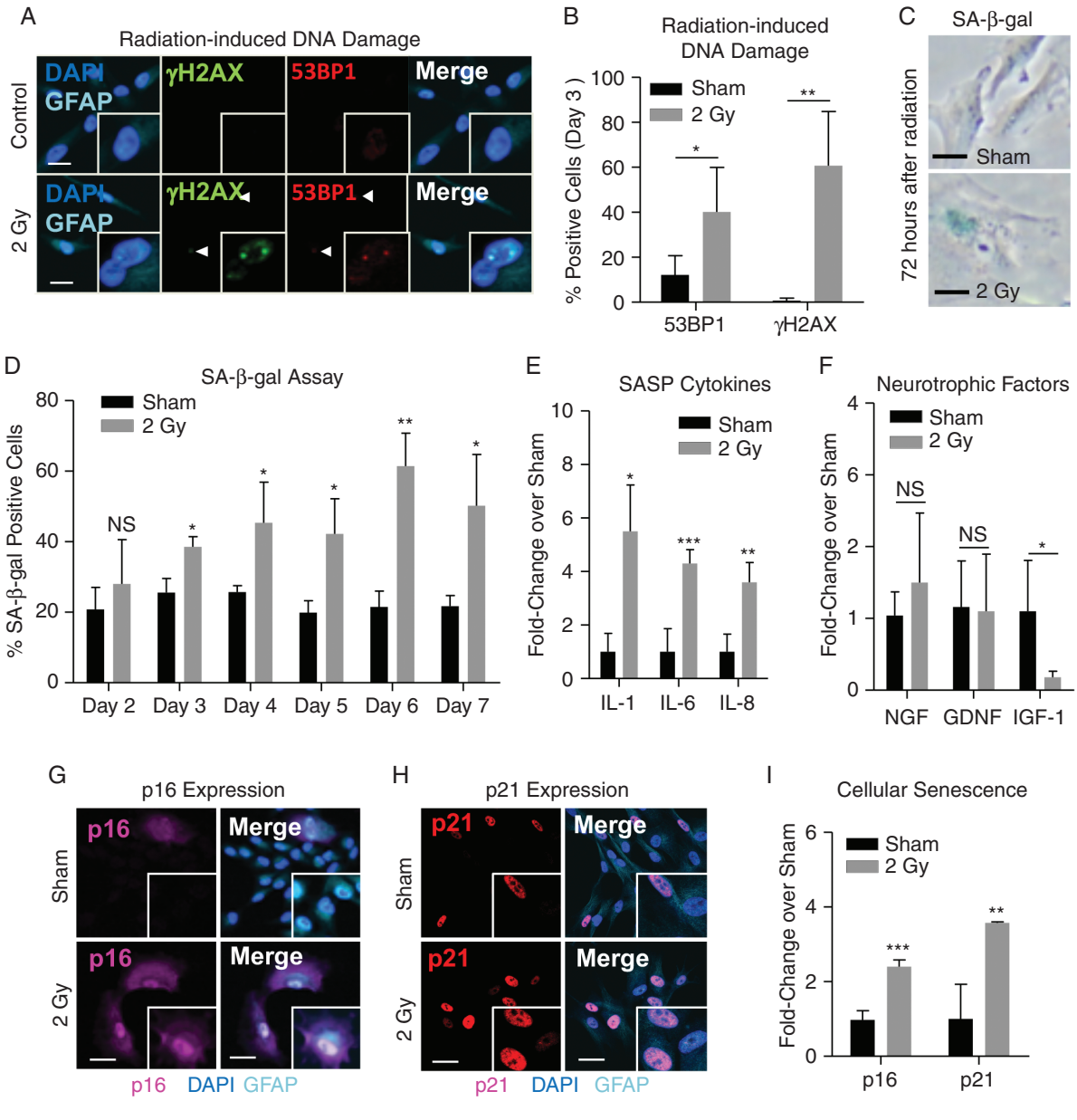

Fig. 2 Radiation induces astrocyte senescence. (A) Representative image and (B) quantification of radiation-induced DNA damage identified by immunolabeling of double-stranded DNA breaks by 53BP1 and γH2AX in primary human astrocytes 3 days after radiation exposure (2 Gy). (C) Representative images of SA-β-gal staining in human astrocytes on day 3 after exposure to radiation (2 Gy). (D) Quantitative summary of the percent of astrocytes with SA-β-gal staining from 2 to 7 days after radiation (2 Gy). (E) Production of SASP-associated cytokine mRNAs (IL-1β, IL-6, and IL-8 mRNA) and (F) neurotrophic factor mRNAs (nerve growth factor [NGF], glial cell–derived neurotrophic factor [GDNF], insulin-like growth factor-1 [IGF-1]) in irradiated or sham-treated primary human astrocytes measured by qRT-PCR (Taqman). Representative images of (G) $p16^{INK4A}$ and (H) $p21^{WAF1}$ immunolabeling in irradiated GFAP-positive human astrocytes on day 6 following exposure to radiation (2 Gy). (I) Quantitation of $p16^{INK4A}$ and $p21^{WAF1}$ immunoreactivity in irradiated (2 Gy) and sham-treated primary human astrocytes on day 6. NS indicates $P > 0.05$, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$ by unpaired 2-tailed Student's *t*-test. Scale = 25 μm.

(Fig. 1, Supplementary Table 3). Quantification of $p16^{INK4A}$-positive astrocytes was completed in 20 microscopic fields (0.5 mm^2) from untreated cancer patients ($n = 4$) and cancer patients receiving cranial radiation treatment ($n = 4$). In addition, 3 patients received stereotactic radiotherapy allowing for comparison of irradiated and untreated regions within the same patient (Fig. 1); these case-matched tissues were further reviewed to identify Hp1γ-positive cell types, to quantify percent of Hp1γ-positive microglia, and to quantify CD68-positive microglia per high-power field (40x) in irradiated and untreated brain tissue (Fig. 1, Supplementary Fig. 3).

Cell Culture

Primary human astrocytes were obtained from Sciencell and maintained in Astrocyte Medium supplemented with 2% fetal bovine serum, 1% astrocyte growth supplement from Sciencell, and 1% penicillin/streptomycin solution. Astrocytes expressed astrocyte-lineage marker (glial fibrillary acidic protein [GFAP]) (Fig. 2A, G–H, Supplementary Fig. 4N–P) were split at a ratio of 1:3 and continued to proliferate through passage 20. All experiments used proliferative, low passage astrocytes (<p10).

The induced pluripotent stem cell (iPSC) line, i20 (NIH stem cell bank), was differentiated to neural stem cells using Gibco Pluripotent Stem Cell Neural Induction (Life Technologies). Mature neurons were differentiated from a transgenic human control iPSC line by neurogenin 2 induction.[21] The iPSCs were plated in Matrigel-coated 10 cm dishes at a density of 1.5×10^6 cells/dish with doxycycline (2 μg/mL) in Dulbecco's modified Eagle's medium/F12 containing N2 supplement (Invitrogen), non-essential amino acids (Invitrogen), L-glutamine (Invitrogen), and Y-27632 (10 μM; Tocris). After 3 days, cells were Accutase treated and plated onto poly-D-lysine/laminin coated 8-chamber slides (Corning) at a density of 300 000 cells in induction media without Y-27632, supplemented with mouse laminin (1 μg/mL; Invitrogen), B27 supplement (Invitrogen), and brain derived neurotrophic factor (10 ng/mL; R+D Systems). Neurons were analyzed after 7 days of differentiation.

Radiation Exposure

Human cells were exposed to ionizing radiation in an X-Rad 320 biologic irradiator (Precision X-ray), at a dose of 0.5 to 20 Gy as indicated.

IL-6 Treatment

Where indicated, recombinant interleukin (IL)-6 (InvivoGen) was incubated with iPSC-derived neural stem cells (NSCs) or mature neurons for 24 hours at a concentration of 5 ng/mL.

Senescence-Associated Beta Galactosidase Assay

SA-β-gal staining was performed with the Senescence Associated (SA)-β-Galactosidase Staining Kit (Cell Signaling Technology).

Transwell Experiments

Human astrocytes were irradiated in the top transwell chamber and co-cultured with untreated neural progenitor cells (NPCs) (ACS-5004) for 96 hours beginning on the third day after irradiation. Additional details are described in the Supplementary Material.

Statistical Analysis

Data are presented as mean and standard deviation of at least 3 independent experiments. Comparisons were made using 2-sided, unpaired Student's *t*-test. Differences were considered significant at $^*P \le 0.05$, $^{**}P \le 0.01$, and $^{***}P \le 0.001$ or NS (not significant).

Additional methods—including antibodies, lentiviral vector transduction, cell viability assay, enzyme-linked immunosorbent assay, quantitative reverse transcriptase (qRT)-PCR, immunohistochemistry, immunofluorescence, western blot, RNA extraction, and cDNA preparation—can be found in the Supplementary Material.

Results

Astrocyte Senescence Is Increased in Irradiated Patient Tissues

Radiation-induced cellular senescence is a stress-induced cell cycle arrest that may contribute to the development of radiotherapy side effects.[18,19] To characterize cellular senescence in the brain, tissue samples from patients with or without a history of radiation treatment were examined (Supplementary Table 1). Immunohistochemistry was performed using antibodies against senescence-associated proteins $p16^{INK4A}$ and Hp1γ[22–24] (Fig. 1A) and scored based on the intensity of cellular labeling (Fig. 1B, Supplementary Table 3). Tissue immunoexpression of senescence proteins was lowest in brain tissue from non-disease, age-matched controls; was increased in untreated cancer patients; and was highest in irradiated tissues (Fig. 1B). Similar results were observed in a subset of patients with the same cancer type (metastatic melanoma; Supplementary Fig. 1A–C) and in patients receiving stereotactic radiotherapy (Fig. 1C–D), which in contrast to non-targeted whole brain radiotherapy allows for comparison of irradiated to untreated brain regions within the same patient as an internal control.

We next aimed to characterize senescent cell types in irradiated patient tissues. Hp1γ- and $p16^{INK4A}$-positive cells were identified by 3 independent pathologists (J.B., B.H., I.O.). The majority of senescence-associated markers co-localized with GFAP-positive astrocytes (Fig. 1E), underscoring the potential importance of astrocyte senescence in the brain's response to radiation. The mild increase in cellular senescence in untreated cancer patient tissues compared with non-disease controls (Fig. 1B) may indicate a role for the tumor microenvironment in promoting reactive astrocytosis and astrocyte senescence, which may be a general response of human astrocytes to injury. However, the number of $p16^{INK4A}$-positive astrocytes was higher in patients receiving radiation treatment compared with untreated cancer patients (Fig. 1F–G), suggesting that radiotherapy may exacerbate this response. In addition, similar numbers of $p16^{INK4A}$-positive astrocytes were observed in tissues irradiated between 3 months and 4 years prior to collection (Supplementary Fig. 2A–B), which is consistent with reports of increased numbers of reactive astrocytes in animal models of radiation-induced brain injury for at least 1 year following radiation exposure.[25,26] Astrocyte senescence is also increased in Alzheimer's disease (Case 7, Fig. 1C–D, Supplementary Fig. 2C–D) and may promote neurotoxicity, highlighting the potential importance of astrocyte senescence in neurodegenerative diseases.[16,17,20] Finally, focal Hp1γ immunoreactivity was

identified in several additional cell types, including microglia, which are important mediators of neuroinflammation[27]; however, this effect was less prominent than the described astrocyte senescence (Fig. 1H, Supplementary Fig. 3A–D).

Radiation Induces Cellular Senescence in Human Astrocytes

Radiation can induce DNA damage either directly through ionization or indirectly through the production of free radicals.[5,14] Adult and pediatric patients with brain cancer may receive 30 to 60 Gy of radiation, which is administered in small doses or fractions of approximately 2 Gy per treatment until the total dose is achieved.[1–3] After a single 2 Gy fraction, primary human astrocytes irradiated in vitro have significant increases in DNA double-strand breaks indicated by γH2AX (P = 0.013) and 53BP1 (P = 0.035) (Fig. 2A–B).[28]

Following accumulation of DNA damage, one of several cell type–specific responses may occur, including induction of apoptosis, mitotic catastrophe, or cellular senescence.[5,15] Our study has identified astrocytes as the major senescent cell type in irradiated brain tissues. In contrast to NSCs

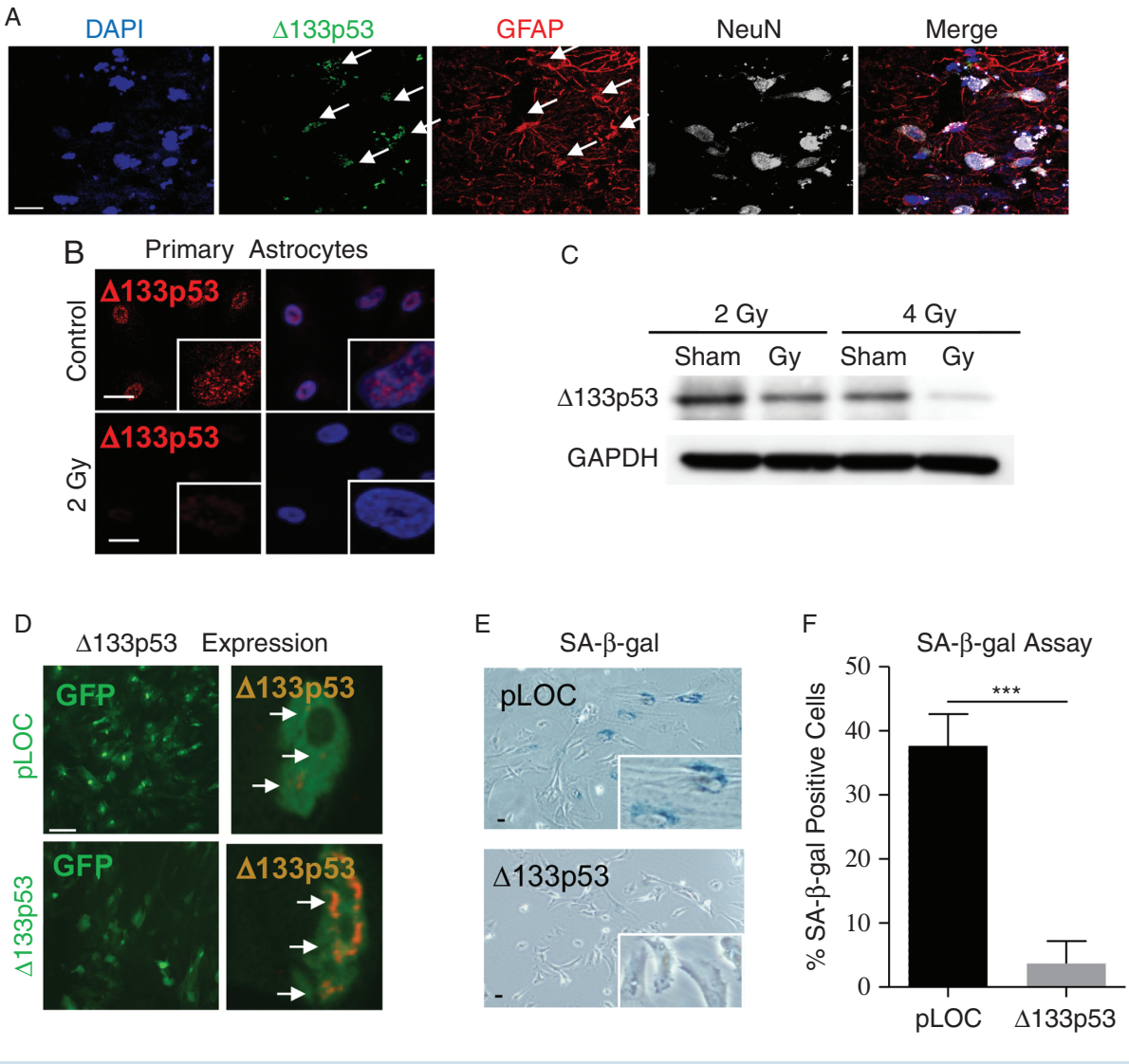

Fig. 3 Δ133p53 is decreased in irradiated astrocytes and its overexpression protects astrocytes from cellular senescence. (A) Non-disease human brain tissue fluorescently labeled with antibodies to Δ133p53, astrocytic GFAP, and neuronal-specific nuclear protein (NeuN) to identify cellular sources of Δ133p53 (arrows). (B) Primary human astrocytes labeled with nuclear staining (4′,6′-diamidino-2-phenylindole [DAPI]) and Δ133p53 on day 6 following either sham or radiation treatment (2 Gy). (C) Western blot analysis of Δ133p53 on day 6 in sham-treated or irradiated primary human astrocytes irradiated one time at 2 Gy or twice at 2 Gy 24 hours apart (fractionated dose, 4 Gy total). (D) Nuclear Δ133p53 expression in human astrocytes transduced 3 days after radiation treatment (2 Gy) with either a green fluorescent protein lentiviral vector driving Δ133p53 expression or its control vector (pLOC). (E) Representative image of SA-β-gal staining in transduced, irradiated human astrocytes (2 Gy) on day 6. (F) Quantitative summary of SA-β-gal staining in primary human astrocytes with lentiviral pLOC and Δ133p53 following radiation exposure (2 Gy). Scale = 25 μm.

(Supplementary Fig. 4A),[15,29] irradiated human astrocytes did not experience induction of apoptosis and maintained over 90% viability for up to 7 days following radiation exposure (Supplementary Fig. 4B–D). To further characterize this, we next investigated astrocytes irradiated in vitro for the induction of cellular senescence, a response that may promote side effects of cancer treatment.[18,19] Irradiated astrocytes experienced a significant, dose-dependent (Supplementary Fig. 4E–G) increase in SA-β-gal staining beginning 2 days after irradiation ($P = 0.010$, 1.5-fold) and persisting for up to 1 week ($P = 0.03$, 2.3-fold) (Fig. 2C–D). SASP-associated cytokines, including IL-1β and IL-6, are known to be upregulated in patients and animal models following radiation treatment.[27,30–33] To determine whether astrocytes may contribute to radiation-induced inflammation, we examined several cytokines implicated in neurodegeneration[20,34] and found a significant increase in IL-1β ($P = 0.016$), IL-6 ($P = 0.0005$), and IL-8 ($P = 0.006$) (Fig. 2E). The significant induction of SASP cytokines in irradiated astrocytes underscores their potential role in promoting neuroinflammation in radiation-induced brain injury. Similar induction of senescence and SASP-associated IL-6 was also observed in astrocytes irradiated at radiosurgical doses (10 Gy; Supplementary Fig. 4H–J). In addition, radiation-induced astrocyte senescence was accompanied by a significant loss of insulin-like growth factor 1 (IGF-1) ($P = 0.015$; Fig. 2F), which is reported to promote astrocyte-mediated neuroprotection and improve neurocognitive function in mouse models of brain injury.[35,36] Finally, irradiated astrocytes demonstrated increased expression of senescence-associated $p16^{INK4A}$ ($P < 0.0001$) and p21 ($P = 0.009$)

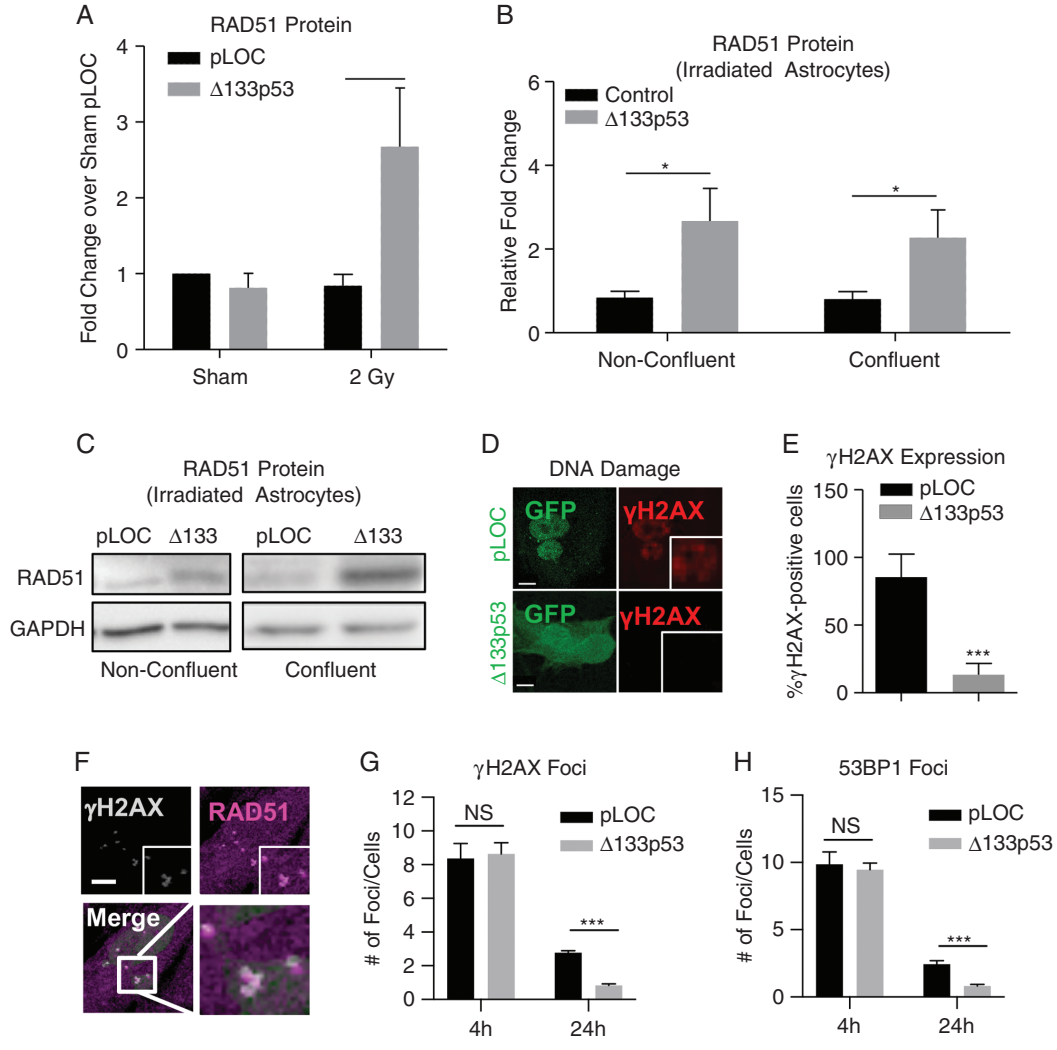

Fig. 4 Δ133p53 promotes DNA repair. (A) RAD51 protein in sham and irradiated astrocytes expressing either control vector (pLOC) or Δ133p53. (B) Quantification and (C) representative western blots of RAD51 protein in irradiated astrocytes expressing pLOC or Δ133p53 at low and high confluency. (D) Labeling of DNA double-strand breaks with γH2AX in transduced, irradiated astrocytes. (E) Quantitative summary of γH2AX staining on day 6 in irradiated human astrocytes transduced with pLOC or Δ133p53 on day 3 after radiation exposure (2 Gy). (F) Representative image of RAD51 and γH2AX labeling 4 hours after radiation exposure. (G) Quantification of γH2AX- and (H) 53BP1-positive foci at 4 and 24 hours after radiation exposure in astrocytes transduced prior to radiation treatment. NS indicates $P > 0.05$, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$ by unpaired 2-tailed Student's *t*-test. Scale = 5 μm.

(Fig. 2G–I), reduced cell number (Supplementary Fig. 4K), enlarged cell size (Supplementary Fig. 4L), increased number of multinucleated cells[15] (Supplementary Fig. 4M), and senescence-associated downregulation of GFAP[37] (Supplementary Fig. 4N–P). Taken together, these in vitro results indicate that irradiated astrocytes undergo senescence, and are consistent with our findings in patient tissues and with animal models of radiation-induced brain injury.[25–28]

Δ133p53 Is Decreased in Irradiated Astrocytes and Its Overexpression Protects Astrocytes from Radiation-Induced Cellular Senescence

Senescent astrocytes are observed in patients with neurodegenerative diseases, including Alzheimer's disease and amyotrophic lateral sclerosis, and have been shown to have reduced expression of the p53 isoform Δ133p53.[20] To identify brain cells expressing Δ133p53 in human brain tissue, immunofluorescence was performed using a Δ133p53-specific antibody, MAP4,[20,38] and cell type–specific antibodies for astrocytes (GFAP-positive[20]) or neurons (NeuN-positive[39]). The majority of Δ133p53 expression co-localizes with GFAP-positive astrocytes (Fig. 3A), indicating that astrocytes are the predominant source of Δ133p53. Following radiation exposure, primary human astrocytes have decreased Δ133p53 (Fig. 3B), which is further diminished after exposure to a second 2 Gy fraction (4 Gy total dose) (Fig. 3C), suggesting that loss of Δ133p53 may be associated with the induction of radiation-induced astrocyte senescence.

As Δ133p53 is diminished in irradiated senescent astrocytes, we investigated whether reconstitution of Δ133p53 expression would protect astrocytes from radiation-induced senescence. First, a lentiviral vector expressing Δ133p53 or pLOC control vector (Supplementary Material) was transduced in primary human astrocytes 3 days after radiation exposure (Fig. 3D). Irradiated astrocytes with reconstituted Δ133p53 had reduced SA-β-gal activity compared with control astrocytes ($P = 0.0006$) (Fig. 3E–F), indicating that Δ133p53 can rescue astrocytes from radiation-induced senescence. Finally, we examined the impact of transducing astrocytes with lentiviral vectors expressing Δ133p53 or pLOC control prior to radiation exposure and found that astrocytes with Δ133p53 had no increase in SA-β-gal staining ($P = 0.483$) compared with an increase of approximately 55% in irradiated pLOC control astrocytes ($P < 0.0001$) (Supplementary Fig. 5A–B), demonstrating that increasing Δ133p53 protects astrocytes from radiation-induced senescence when induced either prior to or after radiation exposure.

Δ133p53 Promotes DNA Repair in Irradiated Astrocytes

Recently, Δ133p53 has been shown to promote DNA repair in fibroblasts from patients with Hutchinson-Gilford

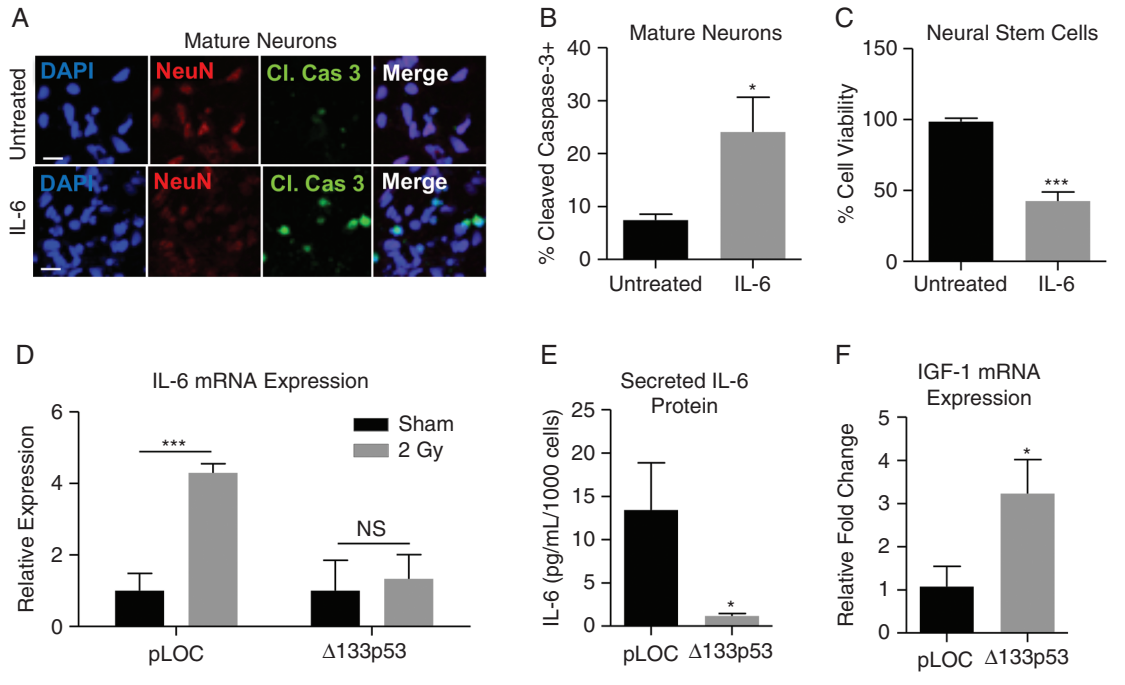

Fig. 5 Δ133p53 regulates radiation-induced, astrocyte-mediated neurotoxicity. (A–B) Immunopositivity of cleaved caspase 3 in mature neurons and (C) viability of neural stem cells following 24-hour IL-6 exposure (5 ng/mL). (D) IL-6 mRNA production in sham and irradiated astrocytes transduced with either Δ133p53 ($P = 0.389$) or the control vector on day 3 and examined on day 6 by qRT-PCR (Taqman). (E) IL-6 protein secreted by astrocytes transduced prior to radiation and examined by enzyme-linked immunosorbent assay, (F) IGF-1 mRNA expression in irradiated astrocytes transduced with pLOC, or Δ133p53 vector prior to radiation exposure ($P = 0.015$) and examined by qRT-PCR (Taqman). NS indicates $P > 0.05$, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$ by unpaired 2-tailed Student's *t*-test. Scale = 25 μm.

progeria syndrome through the promotion of homologous recombination (HR) DNA repair protein RAD51.[40] Following irradiation, RAD51 is significantly increased in astrocytes transduced with Δ133p53 (P = 0.016; Fig. 4A). Although this increase may be due to accelerated cell proliferation,[41] confluent human astrocytes transduced with Δ133p53 maintained a 2- to 3-fold increase in RAD51 (Fig. 4B–C) despite an approximately 3.5-fold decrease in cellular proliferation (Ki-67[42]; Supplementary Fig. 6A–B).The sustained increase in RAD51 at confluency, which is associated with G1 arrest,[43] suggests that the effect of Δ133p53 on HR may be at least in part due to an increased baseline expression of RAD51, although this finding does not rule out an S/G2 phase-specific regulation of HR machinery. To further examine the role of Δ133p53 in HR, DNA double-stranded breaks were labeled with γH2AX. Six days after irradiation, the percent of γH2AX-positive astrocytes was significantly reduced by Δ133p53 transduction after radiation exposure (P < 0.0001; Fig. 4D–E). A similar reduction in γH2AX was also observed in astrocytes transduced with Δ133p53 vector prior to radiation (Supplementary Fig. 6C). To examine DNA repair kinetics at earlier time points, astrocytes were transduced prior to irradiation and labeled at 4 and 24 hours post-irradiation with RAD51, γH2AX, and 53BP1 (Fig. 4F–H, Supplementary Fig. 6D–E). After 4 hours, the number of DNA damage foci labeled by γH2AX and 53BP1 was not significantly different (Fig. 4G–H), suggesting that both control and Δ133p53 transduced cells develop similar levels of radiation-induced DNA damage; however, after 24 hours, Δ133p53-transduced astrocytes had fewer γH2AX (P = 0.00002) and 53BP1 foci (P = 0.0006), suggesting that Δ133p53 promotes DNA repair in irradiated astrocytes.

Δ133p53 Inhibits Astrocyte-Mediated Neuroinflammation

Because radiation-induced brain injury is associated with neurocognitive dysfunction, many studies focus on the effects of radiation on neurons and NPCs.[6,29,44] Secretory factors derived from senescent astrocytes are known to impair astrocyte-mediated neuroprotection in animal models[45] and may promote the late effects of radiation injury by contributing to chronic neuroinflammation. Of the SASP cytokines, IL-6 is most frequently upregulated in neurodegeneration.[46] Following radiation exposure, human astrocytes secrete significantly more IL-6 (P = 0.018; Supplementary Fig. 5C), similar to replicatively senescent astrocytes, which are neurotoxic via IL-6 in neuron-astrocyte co-culture experiments.[20] To examine whether radiation-induced senescent astrocytes are also neurotoxic, we cultured NPCs with irradiated or sham-treated astrocytes separated by a transwell membrane (Supplementary Fig. 5D). In co-culture, there was a significant loss of NPC viability (P = 0.009; Supplementary Fig. 5E) and a 2-fold

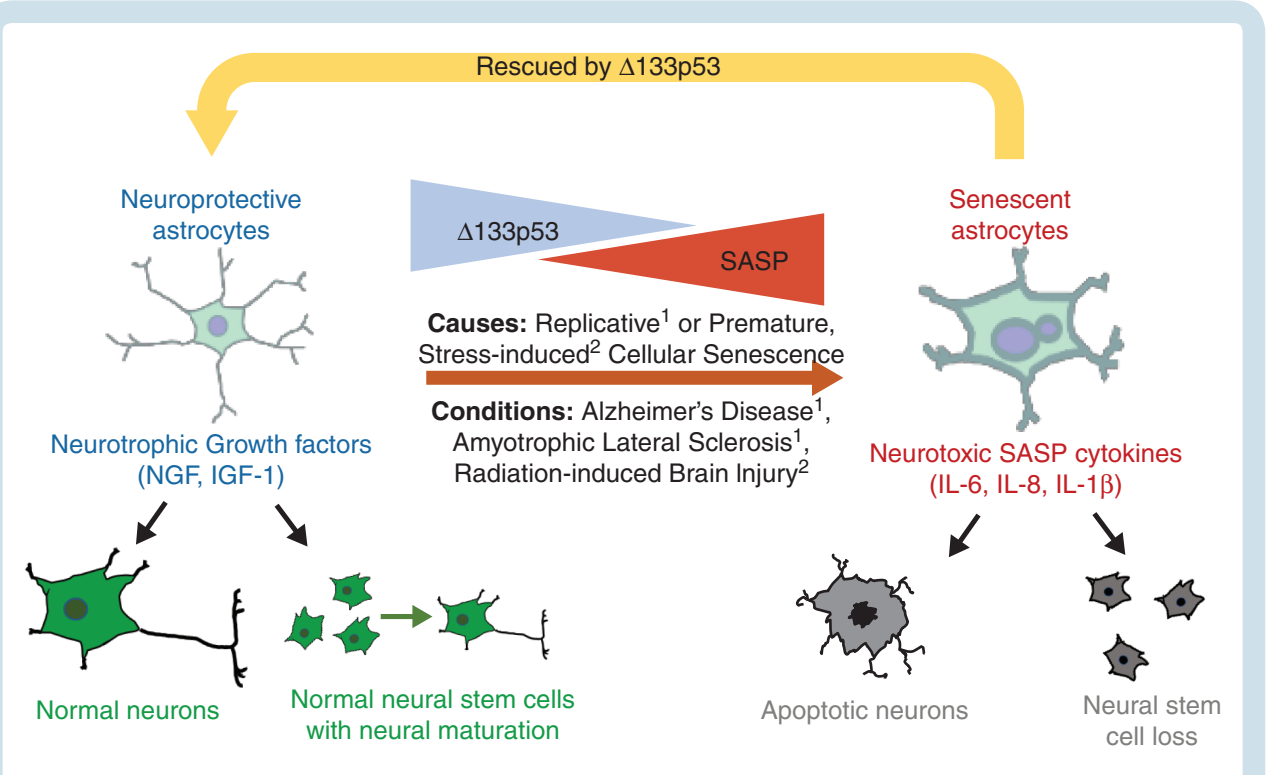

Fig. 6 Proposed model of Δ133p53 regulation of astrocyte-mediated neuroprotection and neuroinflammation. Senescent astrocytes are increased in neurodegenerative diseases, including Alzheimer's disease, and have diminished Δ133p53. Similarly, senescent astrocytes are observed in brain tissues from cancer patients receiving radiation treatment, suggesting that senescent astrocytes may contribute to chronic neuroinflammation in each of these pathologies. These findings are also reproduced in vitro where cellular senescence is induced in irradiated or replicatively exhausted astrocytes and is associated with loss of Δ133p53, adoption of the SASP, and diminished neurotrophic factor production, including IGF-1, which can each be rescued by enhanced expression of Δ133p53. [1]Turnquist et al, 2016; [2]current study.

induction of NPC apoptosis (P = 0.011; Supplementary Fig. 5F–G). These findings are not only consistent with our previous study of senescent astrocyte-mediated neurotoxicity but also demonstrate that astrocytes mediate neurotoxicity through secretory factors such as IL-6,[20] rather than through direct cell-cell contact. This was further examined through direct exposure of NSCs and mature neurons to IL-6 (5 ng/mL). After 24 hours, there was an approximately 10% increase in the percent of mature neurons expressing the apoptotic marker cleaved caspase 3 (P = 0.013; Fig. 5A–B), and the viability of NSCs was reduced to less than 50% (P = 0.0001; Fig. 5C), suggesting that IL-6 plays a causative role in neuronal death mediated by senescent astrocytes.

Because Δ133p53 was found to rescue irradiated astrocytes from senescence (Fig. 3), we next investigated whether Δ133p53 rescues astrocytes from radiation-induced production of neurotoxic IL-6. Irradiated control astrocytes experienced a significant 5-fold increase in IL-6 mRNA measured by qRT-PCR (P = 0.0005). In contrast, IL-6 mRNA was not significantly upregulated in irradiated astrocytes with restored Δ133p53 (P = 0.389; Fig. 5D), indicating that astrocyte-mediated neuroinflammation is repressed by reconstitution of Δ133p53 after radiation treatment. Similar findings were also observed in astrocytes transduced prior to radiation, including a significant reduction in secreted IL-6 (P = 0.017; Fig. 5E). In addition, astrocytes transduced with Δ133p53 demonstrated a partial rescue of neurotrophic IGF-1 mRNA expression (P = 0.015; Fig. 5F). Taken together, these findings suggest that radiation induces astrocyte senescence, thereby promoting astrocyte-mediated neurotoxicity through the production of neurotoxic cytokines. Critically, Δ133p53 has been identified as a potential therapeutic target for inhibiting radiation-induced astrocyte-mediated neurotoxicity (Fig. 6).

Discussion

Radiation-induced brain injury may cause progressive cognitive deterioration, including dementia-like symptoms.[6] It shares pathologic features with aging-associated neurodegeneration, including chronic oxidative stress, inflammation, and reduced neurogenesis.[6,47,48] Current understanding of the pathogenesis of radiation-induced brain injury focuses on the acute loss of NSCs and its effect on hippocampus-dependent functions such as learning and memory.[29,49] However, few studies have addressed the role of astrocytes. Our finding that astrocytes preferentially undergo senescence, while NPCs undergo cell death, indicates that astrocyte SASP may underlie the chronic nature of radiation-induced brain injury.

Animal models of radiation-induced brain injury have identified hypertrophied astrocytes that persist for at least 12 months following radiation treatment.[25,26] Based on our findings in irradiated human tissues and our previous findings in Alzheimer's disease and amyotrophic lateral sclerosis,[20] many of these hypertrophied astrocytes are senescent, an important pathologic characterization that likely extends to other disease processes in the brain.

Following brain injury, astrocytes proliferate as part of reactive astrogliosis, which may lead to replicative senescence.[20,50,51] In addition, direct injury including DNA injury or oxidative damage may induce premature cellular senescence.[17,18,51] Both mechanisms of cellular senescence are controlled by p53 and its isoforms through p53-inducible cell cycle regulators, such as p21.[20,38] In humans, TP53 has at least 12 isoforms through alternative promoters or splicing that may promote or inhibit full-length p53 activities or have independent functions. Of these isoforms, Δ133p53 is the best characterized as an endogenous inhibitor of cellular senescence.[20,38,40] Based on this and previous studies,[40] Δ133p53 enhances DNA repair in senescent cells by promoting HR; however, our study has also demonstrated that expression of Δ133p53 enhanced repair of foci positive for 53BP1, a component of non-homologous end-joining (NHEJ),[52] suggesting that Δ133p53 may also regulate NHEJ in radiation injury by a currently unknown mechanism.

In addition to accumulating DNA damage, senescent cells may promote inflammation through induction of SASP.[16,17] Increased release of the SASP cytokines IL-6[32,33] and IL-1β[33] is reported in animal models of radiation-induced brain injury and may inhibit neurogenesis, contributing to cognitive impairment.[30,53,54] Using anti-inflammatory drugs to target and reduce neuroinflammation in radiation injury improves neurogenesis,[30] while IL-6 has been shown to reinforce radiation-induced senescence in animal models,[55] underscoring the role of chronic neuroinflammation in promoting radiation-induced brain injury. Based on the findings outlined in this study, astrocyte senescence and astrocyte-derived neuroinflammation have been identified as potential contributors to radiation-induced brain injury.

This and previous studies have demonstrated that Δ133p53, through the inhibition of full-length p53, regulates p21,[38,40] RAD51,[40] and IL-6,[20,40] each of which has been shown to be important in radiation-induced injury and neurotoxicity. Although the regulatory interactions between these factors have yet to be elucidated, our findings suggest that induction of the p53 isoform Δ133p53 may have potential therapeutic value by preventing astrocyte senescence and inhibiting astrocyte-mediated neuroinflammation (Fig. 6). Critically, this endogenous isoform is produced in human cells and has not been shown to be mutagenic or oncogenic.[20,38,56] To study the role of Δ133p53 in other cell types and the tumor microenvironment in vivo, ongoing studies seek to establish an animal model and identify compounds which modulate Δ133p53. Future studies aim to reverse the senescence phenotype in diseases, such as radiation-induced brain injury, in which cellular senescence may initiate or worsen disease progression.[20,38,40]

Supplementary Material

Supplementary data are available at *Neuro-Oncology* online.

Keywords

astrocytes | IL-6 | p53 isoform | radiation-induced brain injury | senescence

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