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Interplay of Hormones and p53 in Modulating Gender Dimorphism of Subventricular Zone Cell Number

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Abstract

Previous studies have suggested the existence of a gender bias in repair after demyelination. Here we report the existence of gender dimorphism for the regulation of cell number in the subventricular zone (SVZ), an area that has been studied for its repair potential. The number of $Sox2^+$ multipotential cells in the SVZ of young adult female mice was greater than in age-matched male siblings, but this difference was not evident prior to the surge of sex hormones (i.e., in prepubertal mice). To begin asking whether hormonally derived signals were responsible for these gender-related differences, we analyzed proliferation and survival of cultured male-and female-derived SVZ cells. Estrogen, but not testosterone treatment increased cell proliferation and survival of cultured cells after IFN-γ treatment or after UV irradiation, regardless of the gender of origin. Because apoptosis in UV-irradiated SVZ cells correlated with the expression of the proapoptotic molecule p53, we postulated that this molecule could be responsible for the gender dimorphism in the SVZ. In agreement with this prediction, no difference in the SVZ cell number was detected in male and female *p53* null mice. Together with previous reports, these results implicate *p53* as an important component of the mechanism regulating gender dimorphism in the SVZ.

Keywords

estrogen; subventricular zone; testosterone; apoptosis; cell cycle

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system (CNS), and in MS patients gender dimorphism is well characterized in the progression, risk, and recovery of the disease. Women are more susceptible than men to contracting the disease (Duquette et al., 1992, 1993; Hawkins and McDonnell, 1999; Whitacre et al., 1999); they show a greater number of active lesions on MRI (Pozzilli et al., 2003), but they also tend to have a more favorable clinical course of disease than men (Duquette et al., 1992). These results are paralleled by a greater susceptibility of female mice to develop experimental autoimmune encephalomyelitis (EAE; Voskhul and Palaszynski, 2001). In contrast, men are more often affected by progressive forms of MS (Duquette and Girard, 1993), and they tend to show a greater number of hypointense MRI lesions, indicative of greater tissue damage than in females (Pozzilli et al., 2003).

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Multiple factors can contribute to the explanation of gender dimorphism, including a differential response to sex steroids or intrinsic differences in immune response (Dalal et al., 1997; Wilcoxen et al., 2000; Pelfrey et al., 2002). The reduction in the severity of disease and the decreased relapse rate during pregnancy have further supported the notion that a relationship exists between the hormonal status of the patient and the disease progression (Confavreux et al., 1998; Lorenzi and Ford, 2002; El-Etr et al., 2005). The protective role of estrogens was supported by in vitro studies in cultured rat Schwann cells (Zhu and Glaser, 2008) and rodent (Takao et al., 2004) and human oligodendrocytic lines (Cantarella et al., 2004), whereas testosterone was shown to potentiate death pathways (Caruso et al., 2004).

In vivo studies further revealed gender dimorphism in disease progression and repair and possibly in the response to therapeutic intervention (Pelfrey et al., 2002). The most significant change observed in female mice after ovariectomy was a shift to a more acute progressive EAE course, and gonadectomy did not alter the course of the disease in males (Fillmore et al., 2004). This suggested a protective effect of estrogens that was further supported by the amelioration of the clinical symptoms in male EAE mice after administration of estriol (Palaszynski et al., 2004). The existence of gender-related differences in repair was further suggested by studies on remyelination in old rats with chemically induced demyelination, which revealed a more rapid repair response in females compared with males (Li et al., 2006).

To begin understanding the cellular and molecular substrate of gender differences in repair, we have focused our analysis on cells of the adult subventricular zone (SVZ). These neural stem cells have the rare ability to divide in the adult brain and generate cells of all three lineages, neurons, astrocytes, and oligodendrocytes (Lois and Alvares-Buylla, 1993). SVZ cells have been shown to migrate toward the lesioned areas of the brain (Sundholm-Peters et al., 2005; Kim and Szele, 2008) and affect the efficiency of remyelination in demyelinated lesions (Nait-Oumesmar et al., 1999; Picard-Riera et al., 2002). Previous studies in zebra finch (Lee et al., 2007), mice (Suzuki et al., 2007), and rats (Saravia et al., 2004) suggested that administration of estrogens enhances (Saravia et al., 2004; Lee et al., 2007; Suzuki et al., 2007), whereas ovariectomy suppresses, the proliferation of SVZ cells after injury (Lee et al., 2007; Suzuki et al., 2007). Our study validates previous reports and defines the existence of a gender-based difference in the number of SVZ cells that is associated with a differential response to hormonal treatment and is dependent on the expression levels of the proapoptotic molecule p53.

METHODS AND MATERIALS

Animals

All experiments were performed in postnatal day 8 and postnatal day 60 C57BL/6J mice. We obtained $p53^{-/-}$ mice from Trp53Tm1Tyj C57BL/6J heterozygous breeding pairs. Mouse genotypes were confirmed by tail clipping and PCR using the primers 5′-ACAGCGTGGTGGTACCTTAT-3′ (ImRo36), 5′-TATACTCAGAGCCGGCCT-3′ (ImRo37), and 5′-TCCTCGTGCTTTACGGTATC-3′ (neo), yielding a fragment of 375 bp for $p53^{+/+}$ and 525 bp for $p53^{-/-}$ mice. Mice were maintained under sterile, pathogen-free conditions and in compliance with policies of the Institutional Animal Care and Use Committee of Robert Wood Johnson Medical School/UMDNJ.

Neurosphere Cultures, Treatments, and UV Irradiation

SVZ were dissected from wild-type C57BL/6J mice in Leibovitz's L-15 medium (Gibco, Carlsbad, CA) on postnatal day 60. After digestion with papain (Sigma, St. Louis, MO) at 37° C, cells were mechanically dissociated by pipetting and resuspended in proliferation medium: DMEM/F12 (catalog No. 11039-021; all cultures were maintained in phenol-red-free media;

Gibco) containing 2 mM L-glutamine (Gibco); 25 mM glucose; 2 μg/ml heparin (Sigma); hormone mix including apotransferrin, insulin, putrescine, progesterone, and sodium selenite; and 20 ng/ml epidermal growth factor (EGF). An equal number of cells was plated in uncoated 24-well plates and proliferated in a 37°C, 5% CO_2 incubator for 7 days. During the proliferation, fresh EGF (Gibco) was added every other day.

For hormones and IFN-γ treatment, primary neurospheres were rinsed in 0.1 M PBS and mechanically dissociated by pipetting. The same number of cells was plated in uncoated 48-well plate in proliferation medium with 100 nM of cyclodextrin-encapsulated 17β-estradiol (catalog No. E4389; water soluble; Sigma) or cyclodextrin-encapsulated testosterone (catalog No. T5035; water soluble; Sigma). The hormonal concentration for estrogen or testosterone was calculated on the basis of the actual weight (mg) of the hormone in the preparation. On the following day, 10 ng/ml IFN-γ or PBS as the control was added into each well, and treated cultures were incubated at 37°C, 5% CO_2 incubator. During the culture, media were changed and fresh hormones and IFN-γ were added every other day. After 6–7 days, BrdU incorporation assay was performed with control and treated secondary neurospheres.

For UV irradiation, dissociated cells from primary neurospheres were plated in coated eight-well chamber slides in differentiation medium: DMEM/F12 containing 10% of fetal bovine serum (Gibco) supplemented with 2 mM L-glutamine and 25 mM glucose in the absence and presence of 100 nM estrogen or testosterone. Cells were cultured for 24 hr and then cells exposed to 200 J/m^2 UV with Stratalinker UV cross-linker 1800 (Strata-gene, Los Angeles, CA). Twenty-four hours after UV irradiation, TUNEL assay was performed.

Immunohistochemistry and Immunocytochemistry

For immunohistochemistry, female and male wild-type and p53 null mice, were perfused with 4% paraformaldehyde (PFA) on postnatal day 8 (prepubertal group) and on postnatal day 60 (postpubertal group; young adult) and cut coronally. For immunocytochemistry, cells were fixed with 4% PFA for 20 min at room temperature. Sections and cells were incubated in blocking buffer PGBA (0.1 M phosphate buffer, 0.1% gelatin, 1% bovine serum albumin, 0.002% sodium azide) containing 10% normal goat serum and 0.5% Triton X-100 for 30 min and then stained overnight with primary antibodies in the same blocking buffer. The following primary antibodies were used: anti-Sox2 (1:1,000; catalog No. AB5603; Chemicon, Temecula, CA), BrdU (1:100; catalog No. M0744; Dako, Glostrup, Denmark), and p53 (1:200; catalog No. NCL-p53-CM5p; Novocastra, Newcastle upon Tyne, United Kingdom). After incubation with primary antibodies, sections and cells were incubated with the appropriate secondary antibodies conjugated to fluorescein or rhodamine (Southern Bio-technologies, Birmingham, AL; Amersham Biosciences, Arlington Heights, IL; Jackson Immunoresearch, West Grove, PA; and Vector Laboratories, Bulingame, CA). DAPI (Molecular Probes, Eugene, OR) was used as nuclear counter stain. Images were analyzed using fluorescence microscopy (DM RA; Leica, Heerbrugg, Switzerland) and captured with a Hamamatsu (Hamamatsu City, Japan) CCD camera interfaced with a G4 computer.

BrdU Incorporation Assay and TUNEL Assay

To detect proliferating cells based on their incorporation of BrdU, we labeled secondary neurospheres with 10 μM BrdU (Sigma) for 6 hr, then fixed them with 4% PFA, incubated them in 2 N HCl for 30 min, and washed them with borate buffer (pH 8.5) for 15 min at 37° C. Thereafter, we applied a mouse monoclonal BrdU-specific antibody (1:100; catalog No. M0744; Dako), followed by the appropriate secondary antibody conjugated with fluorophores. Images were captured with a Hamamatsu CCD camera interfaced with a G4 computer. To identify the apoptotic cells, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP end labeling (TUNEL) assay was performed using the Apop-Tag plus kit (Chemicon), following

the manufacturer's instruction. In brief, the cultures were postfixed in pre-cooled ethanol:acetic acid 2:1 for 5 min at −20°C, then incubated with TdT in a humidified chamber at 37°C for 1 hr, followed by the incubation with antidigoxigenin conjugate (fluorescein) at room temperature for 30 min.

Quantification

For quantification of the in vivo experiments, at least three sections per mouse and three mice for each group were evaluated. The total number of $Sox2^+$ cells in the SVZ was counted in both genders on postnatal day 8 and on postnatal day 60. The percentage of $TUNEL^+$ cells was calculated by counting the number of $TUNEL^+$ cells and then dividing this number by the total number of $DAPI^+$ cells. For the count of $BrdU^+$ cells in neurospheres, the total number of $BrdU^+$ cells was counted in a fixed area regardless of the size of the neurosphere. Results were expressed as average ± SD and statistically analyzed via two-tailed Student's tests.

RESULTS

Gender Dimorphism in the Number of SVZ Cells

To define whether a gender bias characterizes the number of SVZ cells that have been implicated in repair (Nait-Oumesmar et al., 1999), we counted the number of multipotential cells identified by Sox2 immunoreactivity in male and female mice before and after puberty. Coronal sections of brains from C57BL/6J female and male mice before (i.e., postnatal day 8) and after (i.e., postnatal day 60) puberty were processed for immunohistochemistry using an antibody specific for Sox2. In the SVZ of prepubertal mice, a similar number of $Sox2^+$ cells was detected (Fig. 1A). In contrast, gender-based differences were observed in the postpubertal group, because the number of $Sox2^+$ cells in the SVZ of young adult females ($n = 97.3 \pm 11.8$) was greater than that in young adult males ($n = 70 \pm 3.3$; Fig. 1B). Together these data suggest the existence of gender dimorphism in the number of SVZ cells that manifests itself only after the hormonal surge.

Effect of Sex Steroids on the Proliferation of SVZ Cells

The fact that the difference in the number of $Sox2^+$ cells between males and females was observed only after puberty implied the potential role of sex steroids in modulating proliferation or apoptosis of SVZ cells. We therefore hypothesized that the gender dimorphism in the number of $Sox2^+$ cells could be attributed to a differential effect of sex hormones in the regulation of proliferation or apoptosis. To test this hypothesis, SVZ cells derived from adult C57BL/6J male or female mice were cultured in suspension as primary neurospheres in phenol-red-free medium, to avoid the potential interference of phenol red, which has been reported to have weak estrogenic activity (Berthois et al., 1986; Bindal et al., 1988). The primary neurospheres were mechanically dissociated, and an equal number of cells was plated in uncoated 24-well plates and cultured in proliferation medium containing either EGF alone or EGF supplemented with 100 nM estrogen or testosterone. After 7 days, cells in S-phase were identified by 6-hr labeling with BrdU (10 μM) followed by immunocytochemistry with antibody against BrdU (Fig. 2A) and cell counting (Fig. 2B). Although we noticed a slightly higher number of $BrdU^+$ cells in female-derived cultures compared with male-derived ones, the difference was not statistically significant (Fig. 2B). We therefore concluded that steroid hormones do not play a major role in modulating the proliferative behavior of SVZ cells under physiological conditions.

Because gender dimorphism has been reported in human and animal models of demyelination characterized by the presence of inflammatory cytokines, we asked whether the subtle differences detected in physiological conditions could be enhanced by the exposure to cytotoxic cytokines. Neurospheres from female- or male-derived SVZ cells were cultured in the presence

of a toxic cytokine (IFN-γ, 10 ng/ml) alone or in the presence of 100 nM estrogen or testosterone (Fig. 2C). The negative effect of IFN-γ treatment on the growth of SVZ cells was indicated by the smaller size of treated neurospheres compared with untreated controls (Fig. 2C). Estrogen treatment counteracted the IFN-γ effect on proliferation, whereas testosterone had no effect (Fig. 2D). BrdU labeling followed by cell counts further revealed a statistically significant difference in the number of proliferating cells between the estrogen-treated and testosterone-treated groups (Fig. 2D), independent of the gender of origin of the culture. Thus, IFN-γ treatment unmasked critical differences of sex steroids in the modulation of SVZ proliferative behavior.

Sex Steroids Modulate the Apoptotic Response of SVZ Cells to UV Irradiation

To further define whether gender and/or hormonal treatment affected the apoptotic susceptibility of SVZ cells, we adopted a model of irradiation, because this stimulus has been widely used to induce apoptosis of SVZ cells in vivo (Amano et al., 2002; Fukuda et al., 2005) and a p53-dependent mechanism of death in several cell types in vitro (Bellamy et al., 1997; Lu et al., 1998; Tang et al., 1998). Briefly, male- and female-derived dissociated SVZ cells pretreated with 100 nM estrogen or testosterone for 24 hr were exposed to UV irradiation [200 J/m^2 UV with Stratalinker UV, as previously reported (Tang et al., 1998)]. After an additional 24 hr in medium supplemented with hormones, apoptotic cells were visualized using the TUNEL assay (Fig. 3A). UV induced apoptosis in 27.5% ± 3.5% of the cells in female-derived cultures and in 31.5% ± 4.9% of the cells in male-derived cultures. Pretreatment with estrogen protected SVZ cells from apoptosis induced by UV irradiation regardless of gender, whereas testosterone had no effect (Fig. 3B). These results indicated a protective effect of estrogen treatment on SVZ cells.

Sex Steroids Modulate the Expression of p53 in UV-Irradiated SVZ Cells

Induction of p53 after UV irradiation is a well-established event in many cell types (Sanchez and Elledge, 1995; Borovitskaya et al., 1996), so we first asked whether a similar phenomenon occurred in SVZ-derived cells. For this reason, dissociated cells derived from the male or female SVZ were exposed to UV irradiation and then processed for immunocytochemistry using antibodies specific for p53 (Fig. 4A). A similar percentage of cells expressed p53 in the absence of any hormonal treatment (Fig. 4B). However, if cells were first pretreated with estrogen or testosterone and then exposed to UV irradiation (Fig. 4C), the percentage of p53-expressing cells was decreased in the estrogen-treated group (Fig. 4D). We conclude that sex steroid, but not gender, differentially modulates the expression of the proapoptotic molecule p53 in response to UV irradiation.

Lack of p53 Abolishes the Gender Dimorphism in the Adult SVZ

We reasoned, based on the differential effect of steroids on apoptosis induced by UV irradiation, that p53 could be a critical determinant of the gender dimorphism detected in the adult SVZ. In other words, we hypothesized that the different numbers of $Sox2^+$ cells in male and female after puberty could be a consequence of the differential activation of p53-dependent pathways of apoptosis in SVZ cells that are differentially affected by sex steroids. To test this hypothesis, male and female p53 null mice were perfused on postnatal day 60 (post-pubertal group), and coronal sections of the brains were processed for immunohistochemistry using an antibody against Sox2 (Fig. 5A). In agreement with the original prediction, immunohistochemical analysis of sections from male and female p53 null mice revealed no difference in the number of $Sox2^+$ cells between the two genders (Fig. 5B). Thus, the difference in $Sox2^+$ cells between males and females that was observed in young adult mice was completely abolished in p53 knockout mice. Taken together, our results suggest an important role of sex steroid and p53 in modulating gender dimorphism of SVZ cell number.

DISCUSSION

This study presents new data supporting a proliferative and protective role of estrogens in SVZ cells and suggests that the gender dimorphism in SVZ cell number likely is the result of the interplay between estrogen and negative testosterone effects on p53-mediated pathways in the SVZ. Several studies have supported the importance of SVZ cells in remyelination after demyelination induced by lysolecithin injection (Nait-Oumesmar et al., 1999), cuprizone diet (Mason et al., 2000Mason et al., 2004), and EAE (Picard-Riera et al., 2002). SVZ cells isolated from the neonatal and adult brain have also been shown successfully to remyelinate demyelinated lesions after transplantation in the brain and the spinal cord of mice with EAE (Ben-Hur et al., 2003; Einstein et al., 2003; Pluchino et al., 2003) or in proximity to lesions induced by ethidium bromide injection (Keirstead et al., 1999; Smith and Blakemore 2000). In all these instances, neonatal neurospheres were able to differentiate into OLs and make new myelin, although at low efficiency (Keirstead et al., 1999). Similarly, adult neurospheres, obtained from either human or rodent tissue, have been shown to generate new myelin when transplanted into the nonmyelinated brain of the mutant *"shiverer"* mouse (Lachapelle et al., 2002) or the spinal cord of ethidium-bromide injected mice (Akiyama et al., 2001) or when injected intracerebrally or into the blood stream of mice with EAE (Pluchino et al., 2003).

In this study, we have identified the existence of a gender-related difference in the SVZ cell number, which is unmasked by the presence of inflammatory cytokines. We report here that postpubertal female C57Bl/6 mice have a greater number of $Sox2^+$ multipotential progenitors than male siblings and that this difference is the likely consequence of hormonal effects, insofar as it is detected only in young adults and not in prepubertal animals. Our data are consistent with previous reports suggesting that administration of estrogens enhances proliferation of SVZ (Saravia et al., 2004; Lee et al., 2007; Suzuki et al., 2007). However, our research differs from the conclusions of other studies in estrogen-treated adult neurospheres, which suggested that 17β-estradiol treatment decreased proliferation in vitro (Brannvall et al., 2002). We believe that the discrepancy with the latter study is the consequence of the distinct experimental conditions. It is important to mention that in our study the effect of estrogen treatment in vitro was not detected when the cells were maintained under physiological conditions but only after the cells were challenged with damaging stimuli (i.e., IFN-γ treatment or UV irradiation). This is consistent with the in vivo reports for rodents, when proliferation was measured in mice or rats after injury (Saravia et al., 2004; Suzuki et al., 2007). Also, in zebra finch brain, the increased level of cell proliferation was detected in the SVZ only after injury, and this proliferative response was suppressed by ovariectomy and recovered with the replacement of estrogen (Lee et al., 2007).

Estrogens have also been shown to protect various cell types from distinct types of death. In oligodendrocytes, for instance, 17β-estradiol protects from death induced by oxidative stress (Takao et al., 2004; Cantarella et al., 2004), whereas testosterone has been shown to potentiate death pathways, by synergistic activation of p53-dependent gene expression (Caruso et al., 2004). Our results clearly indicate that a similar phenomenon occurs in SVZ cells after UV irradiation. Whereas estrogen pretreatment protects from apoptosis, testosterone has no affect and the death correlates with the expression of the proapoptotic molecule p53.

We have previously reported the importance of p53 in regulating the cell cycle of SVZ cells (Gil-Perotin et al., 2006) and in modulating the response to cuprizone-induced demyelination (Li et al., 2008). In this study, we have identified p53 as an important downstream target of testosterone and therefore as a critical modulator of the gender-based difference in SVZ cell behavior. The role of p53 in gender dimorphism has been proposed in the maturation of bone marrow cells (Gupta and Singh, 2007) and in tumor growth of lymphoma cells (Gupta and Singh, 2008) but not in the central nervous system. In addition, anecdotal information on the

viability of p53 null embryos has indicated that females have a higher propensity to die from exencephaly. Together, these data support the idea that cell number regulation is the result of a complex interplay between extrinsic influences (i.e., hormones, growth factors) and intrinsic determinants (i.e., cell cycle molecules, such as p53).

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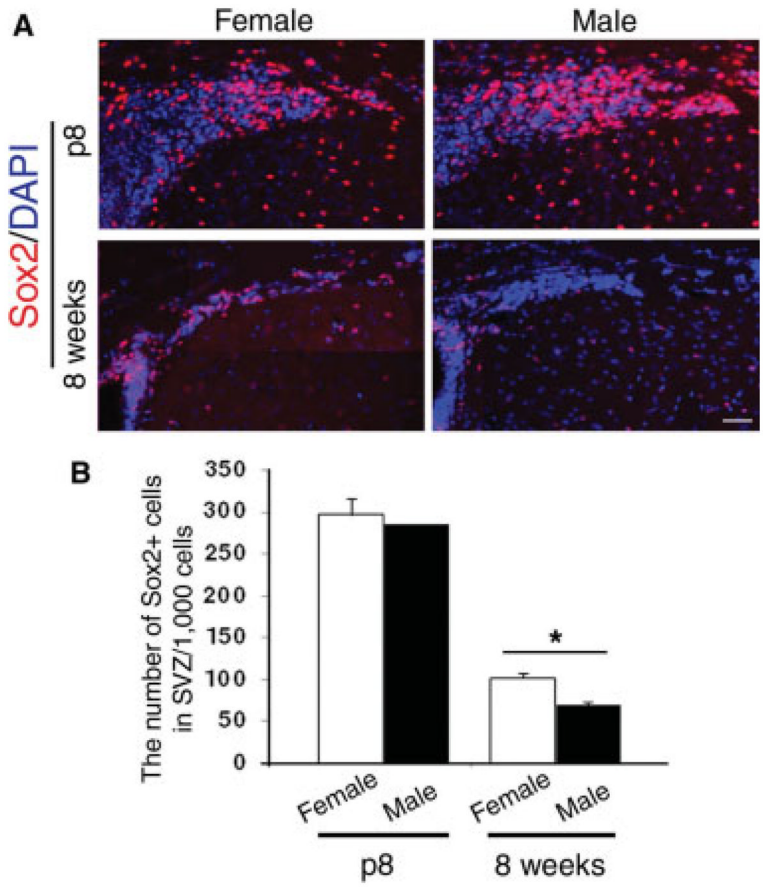

Fig. 1.

Gender dimorphism in the number of multipotential SVZ cells. **A**: Composite image of multiple photograms of coronal sections of wild-type male (n = 3) and female (n = 3) mice at postnatal days 8 (p8) and p60 (8 weeks). Immunohistochemistry for Sox2 (red) and DAPI (blue) was used to identify the multipotential cells in the SVZ. Note that the number of $Sox2^+$ cells clearly shows an age-dependent decline that is much more pronounced in males. **B**: Bar graphs indicate the average number of $Sox2^+$ cells counted per 1,000 cells in the SVZ of mice of the indicated gender and age. The error bars indicate SD. $^\star P < 0.05$. Scale bar = 100 μm.

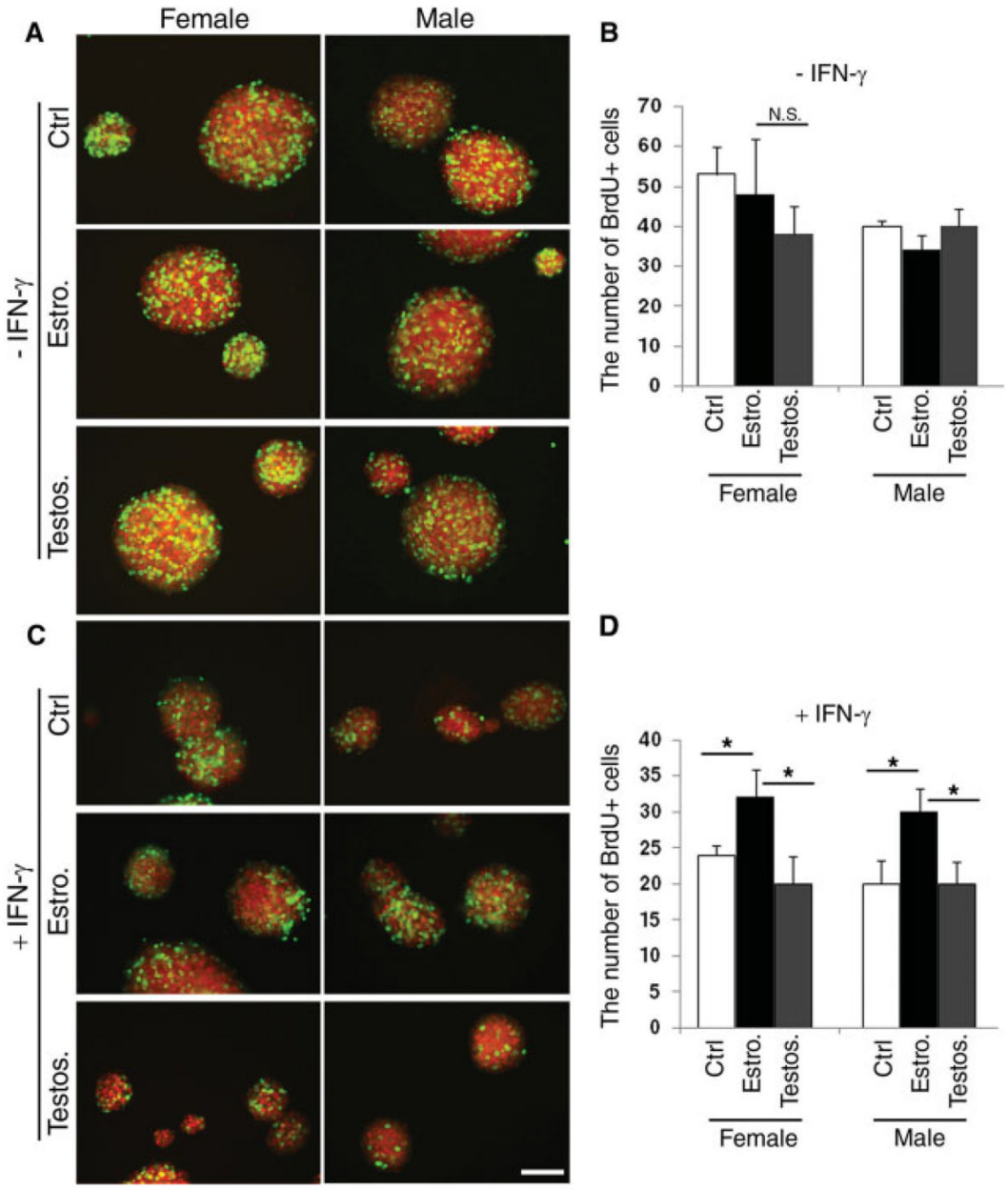

Fig. 2.

The proliferative response of SVZ cells to hormonal treatment is enhanced by the presence of cytotoxic cytokines. **A**: Confocal image of secondary neurospheres generated by mechanically dissociating primary neurospheres and growing the same numbers of cells in medium supplemented with EGF alone (ctrl) or with 100 nM estrogen (Estro) or testosterone (Testos) for 7 days. The cells in S-phase were labeled with a 6-hr pulse of BrdU and identified by immunocytochemistry using specific antibodies for BrdU (green) and Sox2 (red). **B**: Bar graphs represent the results of the quantification of $BrdU^+$ cells in secondary neurospheres cultured as described in A. Bar shows average of three experiments ± SD. **C**: Confocal image of secondary neurospheres generated by mechanically dissociating primary neurospheres and growing the same numbers of cells in medium supplemented with EGF + 10 ng/ml IFN-γ alone (ctrl) or with 100 nM estrogen (Estro) or testosterone (Testos) for 7 days. Note the dramatic

effect of IFN treatment on the size of the male-derived neurospheres. **D**: Bar graphs represent the results of the quantification of $BrdU^+$ cells in secondary neurospheres cultured as described in C. Bar shows average of three experiments ± SD. *$P < 0.005$. Scale bar = 100 μm.

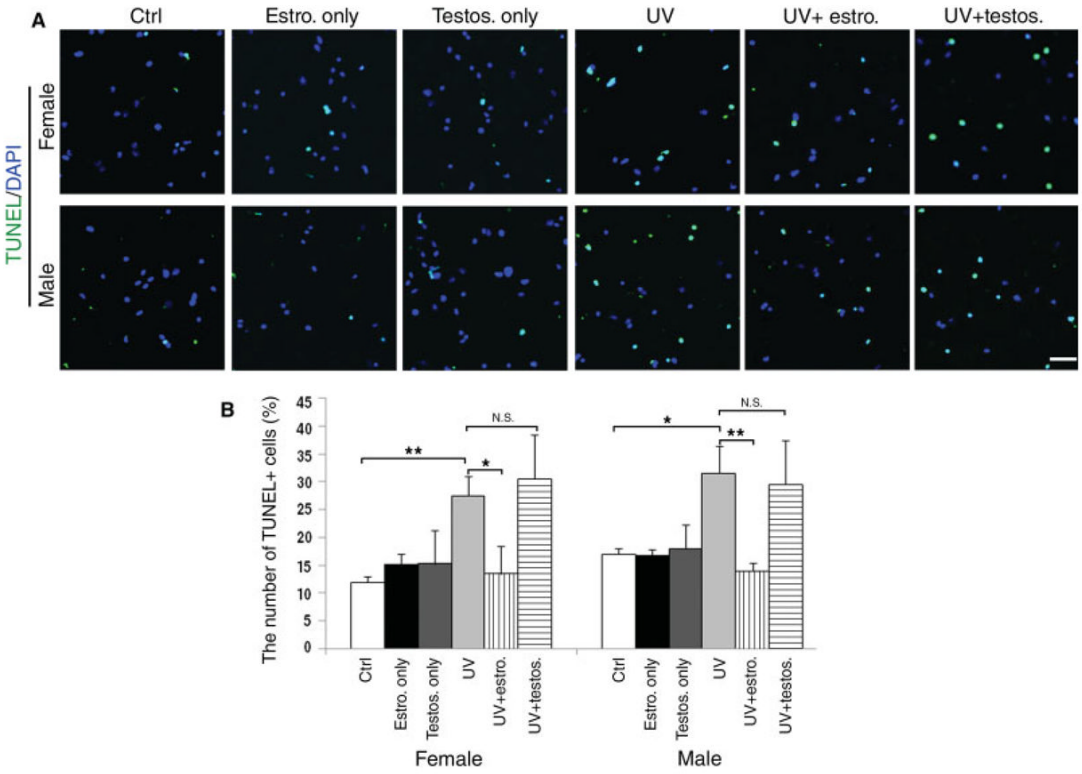

Fig. 3.

Sex steroid hormones affect apoptotic response to UV irradiation in SVZ cells in vitro. **A**: Immunofluorescence of dissociated SVZ cells that were either treated with hormones or first pretreated with 100 nM estrogen or testosterone for 24 hr and then UV irradiated (200 J/m^2). Apoptosis was assessed by TUNEL (green) after an additional 24 hr under the same culture conditions. Nuclei identified by DAPI (blue). **B**: Bar graph indicates the percentage of apoptotic cells relative to the total number of DAPI$^+$ cells in each condition. Bar graphs show average of three experiments ± SD. ★$P < 0.1$, ★ ★$P < 0.05$; N.S., not significant. Scale bar = 100 μm.

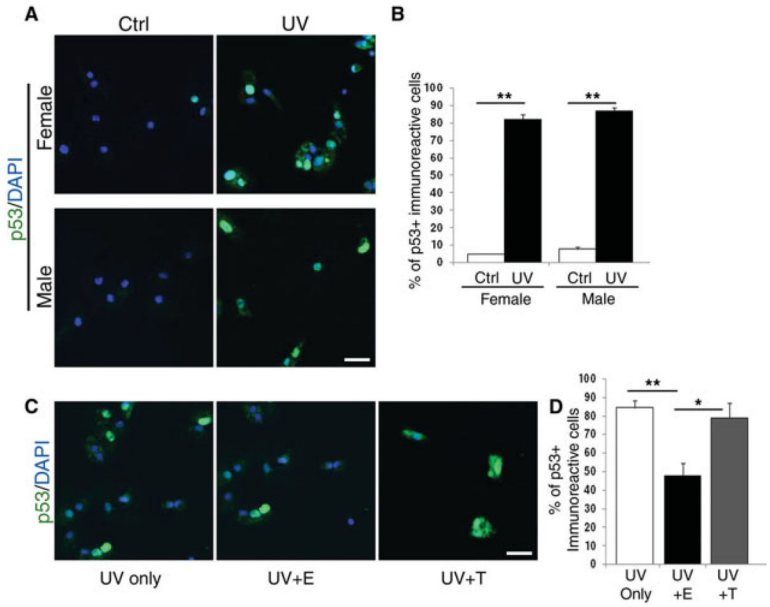

Fig. 4.

Differential modulation of UV-induced p53 expression in SVZ cells in response to hormonal treatment. **A**: Immunofluorescence micrograph of dissociated SVZ cells either untreated (Ctrl) or exposed to 200 J/m^2 UV irradiation (UV) and processed for immunocytochemistry with antibodies recognizing p53 (green). DAPI (blue) was used as nuclear counterstain. **B**: Bar graph indicates the percentage of p53$^+$ cells relative to the total number of DAPI$^+$ nuclei. Bar graphs show mean of three experiments ± SD. **C**: Cells were then irradiated either in the absence (UV only) or in the presence of either 100 nm estrogen (UV + E) or 100 nm testosterone (UV + T) and then stained with p53-specific antibodies. **D**: Bar graphs indicate the percentage of p53$^+$ cells relative to the total number of DAPI$^+$ nuclei in each condition. Bar graphs show average of three experiments ± SD. ★ $P < 0.05$, ★ ★$P < 0.005$. Scale bars = 50 μm.

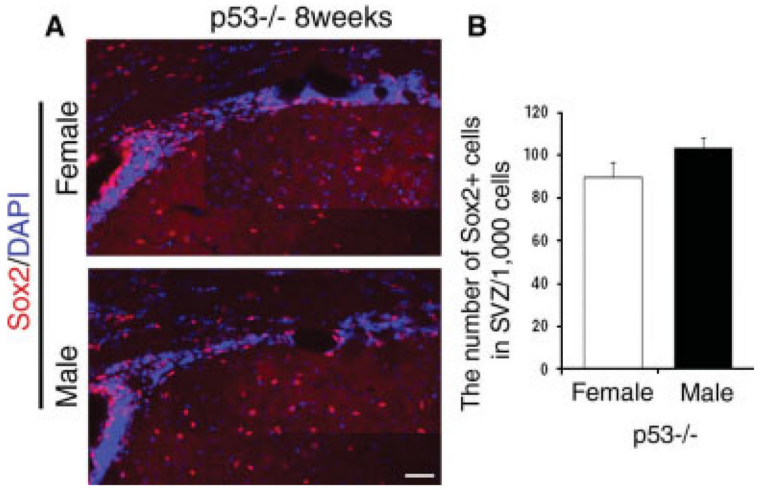

Fig. 5.

The gender dimorphism in the number of $Sox2^+$ cells in the adult SVZ is not detected in the absence of p53. **A**: Composite image of multiple photograms of coronal sections of p53 knockout male (n = 3) and female (n = 3) mice at postnatal day 60. The brain sections were processed for immunohistochemistry using an antibody specific for Sox2 (red). DAPI (blue) was used as nuclear counterstain. **B**: Bar graphs indicate the average number of $Sox2^+$ cells counted per 1,000 cells in the SVZ of p53 null mice of the indicated gender. The error bars indicate SD. Scale bar = 100 μm.