

Original Article

HspB5 protects mouse neural stem/progenitor cells from paraquat toxicity

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Abstract: Introduction: HspB5 (α B-crystallin) is known to be involved in a variety of cellular functions, including, protection of cells from oxidative damage and inhibiting apoptosis. Neural stem/progenitor cells (NSPCs) have significant therapeutic value, especially in the NSC/NPC transplantation therapy. However, the viability of the transplanted NSPCs remains low because of various factors, including oxidative stress. Objective: The current investigation explored the possible role of HspB5 in the protection of mouse NSPCs (*mNSPCs*) against paraquat-induced toxicity. Methods: The recombinant human HspB5 was expressed in *E.coli* and was purified using gel filtration and Ion-exchange chromatography. The biophysical characterization of HspB5 was carried out using DLS, CD, and Analytical Ultracentrifugation (SV); the chaperone activity of HspB5 was determined by alcohol dehydrogenase aggregation assay. We have subjected the *mNSPCs* to paraquat-induced oxidative stress and monitored the protective ability of HspB5 by MTT assay and Hoechst-PI staining. Furthermore, increase in the expression of the anti-apoptotic protein, procaspase-3 was monitored using western blotting. Results: The recombinant HspB5 was purified to its homogeneity and was characterized using various biophysical techniques. The externally added FITC-labeled HspB5 was found to be localized within the cytoplasm of *mNSPCs*. Our Immunocytochemistry results showed that the externally added FITC-labeled HspB5 not only entered the cells but also conferred cytoprotection against paraquat-induced toxicity. The protective events were monitored by a decrease in the PI-positive cells and an increase in the procaspase-3 expression through Immunocytochemistry and Western blotting respectively. Conclusion: Our results clearly demonstrate that exogenously added recombinant human HspB5 enters the *mNSPCs* and confers protection against paraquat toxicity.

Keywords: Neural stem/progenitor cells, α B-crystallin, HspB5, paraquat, cytoprotection

Introduction

Neural stem cells (NSCs) are self-renewing, multipotent cells derived from the ectoderm during the early embryonic stages; they give rise to neurons and glial cell types [1]. NSCs were isolated from different parts of the brain [2] and can be cultured as Neurospheres, which mimic the neurogenic niche [3, 4]. Neurospheres are three-dimensional floating aggregates containing Neural Stem Cells/Neural Progenitor Cells (NSPCs) [5].

NSC transplantation has opened new avenues in the field of Neurosurgery. It is a promising therapeutic approach and has shown beneficial effects, such as improving the neural microen-

vironment and cognitive behavior in various animal models of Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and other neurodegenerative diseases [6]. However, the transplanted NSCs have a poor survival rate in the new and hostile environment, majorly due to oxidative stress. Exposure to oxidative reagents such as H_2O_2 drastically reduced the cell viability in cultured rat NSCs [7] and adult spinal cord-derived NSCs/neural progenitor cells [8]. Increasing the survival of NSCs would be beneficial, especially in case of NSC transplantation therapy.

Paraquat is used globally as an herbicide. It is used in many laboratory studies to induce oxidative stress. Several mechanisms are pro-

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posed to explain the cytotoxicity of paraquat [9]. However, the consensus is that paraquat induces oxidative stress, leading to an increase in ROS, ultimately leading to cellular damage and apoptosis [9, 10].

Heat shock proteins (Hsps) protect cells from oxidative stress [11]. Heat shock, prior to the exposure of paraquat, rescues the immortalized rat brain neuroblasts (E18) from cell death; the heat shock response leads to elevated levels of HspB1 and Hsp70, which directly correlates with cellular protection [12].

Heat shock proteins bind to non-native proteins and prevent aggregation and the consequent cellular damage. In addition, they also exhibit chaperone activity, help in many cellular processes and confer cytoprotection against environmental stresses viz. heat stress, oxidative stress, chemical stress, etc., [13]. Among Hsps, small Hsps (sHsps) are a group of heat shock proteins characterized by the presence of an alpha-crystallin domain (ACD) in their structures; their monomeric molecular sizes range between 12 kDa to 43 kDa [14].

α B-crystallin (HspB5), one of the member proteins of the sHsp family is found in many tissues and is also reported to be present at low concentrations in the extracellular space/fluid. Earlier studies have shown the translocation of HspB5 from the cytoplasm into the nucleus upon stress. The reason for HspB5's nuclear translocation is unclear, but it interacts with proteins such as intranuclear lamin A/C and splicing factor SC-35 [15]. HspB5 works majorly as a molecular chaperone that binds to misfolded proteins and prevents them from aggregation [14]. HspB5 is shown to be associated with stress-induced apoptosis, cell differentiation, cell cycle, cancer, and angiogenesis [14, 16]. HspB5 has a protective role in CNS conditions such as Alzheimer's disease, multiple sclerosis, cerebral ischemia, and Alexander disease [17-21]. HspB5 protects the cells from apoptosis induced by oxidative agents such as H_2O_2 , drugs such as Staurosporine, and cytokines such as TNF- α [14, 16]. Earlier findings demonstrated that HspB5 protects myoblasts against TNF α -induced cytotoxicity by activating NF κ B [22]. Brownell et al. have listed the role of HspB5 in neurological disease and its neuroprotective characteristics [23]. Cell penetration peptide (CPP) tagged recombinant HspB5 pro-

tections the human lens epithelial cells from oxidative stress and heat-induced cell death [24].

Intravenously administered recombinant HspB5, in a spinal cord injury (SCI) mouse model, was shown to be taken up by oligodendrocytes and glial cells at the injury site and help in the recovery [25]. Intravenously administered recombinant HspB5 is known to promote axonal regeneration [26], rescue the optic nerve oligodendrocytes [27], and increase the survival of retinal ganglion cells (RGC) in optic nerve crush experiments in rats [28].

In the current study, we have investigated the protective ability of exogenously added recombinant human HspB5 in the context of *m*NSPC. Our results demonstrate that exogenously added HspB5 not only gains entry into the cultured *m*NSPCs but also confers protection against paraquat-induced apoptosis. Such protective ability might prove useful in NSC transplantation therapy.

Material and methods

Overexpression, purification, and FITC-labeling of human recombinant HspB5

Human recombinant HspB5 was purified using the procedure described earlier [8]. The protein was then buffer exchanged from 1xTNE to PBS (20 mM phosphate buffer with 100 mM NaCl, pH 7.4). After purification, the protein was labeled with fluorescein isothiocyanate (FITC) (F7250, Sigma). Briefly, 50 μ L of 1 mg/mL FITC was added to 1 mL of protein solution (1 mg/mL) and incubated overnight at 4°C under constant stirring. The FITC-tagged protein was separated from the untagged protein by using a Sephadex G-25 desalting column. The protein concentration was determined using the extinction coefficient of HspB5 (1 mg/mL protein gives an OD of 0.693 at 280 nm). The purified and labeled proteins were stored at 4°C until further experiments were carried out.

Biophysical characterization

Dynamic Light Scattering (DLS): Photocor DLS Instrument (Photocor Instruments Inc., College Park, MD) fitted with a 633 nm, 25 mW laser was used for the determination of the hydrodynamic radii (R_h) of HspB5 (1 mg/mL) in 20 mM phosphate buffer with 100 mM NaCl, pH 7.4 at

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25°C by measuring the scattering at 90° angle. Protein samples were filtered through a 0.22 µm syringe filter before the experiment. DYNALS v2.0 software was used to analyze the data.

Circular dichroism (CD): Far UV-CD spectra of the protein (0.2 mg/mL protein in a 0.05 cm pathlength cuvette) were recorded at room temperature at a step size of 0.2 nm and an approximate scan time of 5 min using an Applied Photophysics instrument, Chirascan Plus. An average of four accumulations was reported as the final spectra. The spectrum was recorded from 195 nm to 260 nm. All the ellipticity values obtained were transformed to mean residue mass ellipticity.

$$[\theta]_{\text{MRM}} \text{ (deg. cm}^2 \cdot \text{dmol}^{-1}\text{)} = \theta \times \text{MRW}/10cl$$

θ : Ellipticity in mdeg; MRW: mean residue weight; c: Concentration in mg/mL, and l: path length in cm.

Sedimentation-velocity measurements: Sedimentation-velocity (SV) measurement was performed using an Optima XL-I analytical ultracentrifuge (Beckman Coulter, Fullerton, CA, USA). A 1 mg/mL (50 µM) sample of HspB5 in 20 mM phosphate buffer, pH 7.4, containing 100 mM NaCl, was subjected to centrifugation at 24,000 rpm at 20°C in a rotor. The sample was scanned for absorbance at 280 nm. The data were analyzed using the 'SEDFIT' program, and the sedimentation coefficient S_{20w} and the molecular mass of the protein were calculated as described earlier [29, 30]. 'SEDFIT' uses nonlinear regression fitting of the sedimenting boundary profile with the Lamm equation.

$$\frac{dc}{dt} = \left(\frac{1}{r}\right) \cdot \left(\frac{d}{dr}\right) \cdot \left[\left(rD \frac{dc}{dr}\right) - s\omega^2 r^2 c\right]$$

$c(r, t)$ stands for concentration distribution of a species with diffusion coefficient D and sedimentation coefficient s , and $\omega^2 r$ represents the centrifugal field.

Chaperone assay: In this study, we have used the thermal aggregation of alcohol dehydrogenase (ADH; 0.2 mg/mL in 20 mM phosphate buffer with 100 mM NaCl, pH 7.4) as a model to study the chaperone-like activity of HspB5. The aggregation of the target protein at 48°C, either in the absence or in the presence of HspB5 (0.0125 mg/mL), was moni-

tored as right-angle light scattering using a Hitachi F-7000 fluorescence spectrophotometer. The cuvettes containing the samples were placed in a thermostated-cuvette holder; the excitation and emission wavelengths were set at 465 nm, and the excitation and emission bandpass were set at 2.5 nm. The response time was set at 2 seconds, and the light-scattering was measured as a function of time.

Isolation and maintenance of mNSPCs

Pregnant female BALB/c mice were used to obtain embryonic day 14 (E14) embryos (from CCMB animal house facility (IEC 117/2016)). The mice were euthanized by cervical dislocation, and E14 embryos were collected. The cortical, hippocampal, and striatal tissues were aseptically dissected from fetal brains and were homogenized by passing them through a sterile Pasteur pipette several times. The detached cells were further centrifuged at 1,000 g, and the cell pellet was resuspended in NeuroCult™ NSC Basal Medium (05700, Stem cell technologies) supplemented with NeuroCult™ NSC Proliferation Supplement (05701, Stem cell technologies) and 20 ng/mL epidermal growth factor (EGF) (E4127, Sigma). The cultures were then maintained in a humidified atmosphere with 5% CO₂ at 37°C, with media replacement every 3-4 days until healthy neurospheres were obtained. The *mNSPCs* were stained for stem cell markers.

Localization of exogenously added HspB5 in mNSPCs

In order to study whether the *mNSPCs* take up the exogenously added HspB5, the *mNSPCs* were seeded on poly L-Ornithine (0.1 mg/mL; Sigma P4707) -coated coverslips and incubated with different concentrations of FITC-tagged protein for 24 hours. After incubation, the coverslips were taken, washed with PBS, counterstained with DAPI, and mounted on to grease-free slides; the slides were then analyzed by confocal microscopy.

Anti-apoptotic activity of HspB5 against paraquat-induced oxidative stress

To study the protective effect of HspB5 against the oxidative stress-induced apoptosis, the *mNSPCs* were seeded on poly L-Ornithine (0.1 mg/mL; Sigma P4707) -coated plates and pre-

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treated with HspB5 (100 µg/mL) for 24 hours and then treated with 3 mM paraquat (PQ²⁺) for 4 hours.

Hoechst-propidium iodide staining: Hoechst 33258 dye (Sigma) and propidium iodide (PI; Sigma) staining was used to evaluate the cell viability of paraquat-treated *m*NSPCs (PQ²⁺), HspB5 pre-treated *m*NSPCs exposed to paraquat (HspB5-PQ²⁺), and control *m*NSPCs. In brief, Hoechst dye was added to the culture medium (10 µg/mL final concentration), and the samples were incubated at 37°C for 30 minutes. PI (50 µg/mL final concentration) was added immediately before imaging with a fluorescence microscope. Hoechst-positive + PI-positive cells were counted as dead cells, and Hoechst-positive + PI-negative cells were counted as live cells.

MTT assay: 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT; Sigma) assay was used to assess the metabolic activity of *m*NSPCs at 4 hours after paraquat treatment. For the assay, *m*NSPCs (control, PQ²⁺, HspB5-PQ²⁺) were incubated with MTT (0.5 mg/mL) and incubated for 3 hours at 37°C. Following incubation, the MTT solution was removed, and 100 µL of DMSO was added to dissolve formazan crystals. Absorbance was measured at 560 nm using a microplate reader (Synergy H1 Hybrid multi-mode plate reader; Gen5 software).

Immunocytochemistry: The cells were fixed with 4% paraformaldehyde in 0.1 M PBS, pH 7.4, for 30 minutes. After the PBS wash, the cells were permeabilized with 0.5% Triton X-100 in PBS for 10 minutes. Non-specific sites were blocked with 2% bovine serum albumin (SRL) for 1 hour at room temperature. Appropriate primary antibodies were added and incubated overnight at 4°C. After PBS washes, the secondary antibodies were added and incubated for 2 hours at room temperature. After washing with PBS, the cells were counterstained with DAPI and visualized using a fluorescence microscope. Primary antibodies used were Caspase-3; 1:250; Cell Signaling Technologies - 9915S, HspB5; 1:200; Enzo life sciences - ADI-SPA-223. Secondary antibodies used were anti-rabbit/mouse; 1:500; conjugated with Alexa Fluor 594; Life Technologies - A11037/A11005.

Western blotting: The cells were lysed with RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 8.0, Protease inhibitor cocktail), and the concentration of proteins was determined by Bradford method. Equal amounts of protein (50 µg) from all the samples were loaded onto a 12% polyacrylamide gel and were electrophoresed. Further, the proteins on the SDS-PAGE gel were blotted onto a PVDF membrane using an ECL Semi-dry blotter (TE 77 Semi-dry transfer unit, Amersham Biosciences). The membrane was blocked with 5% BSA solution for 1 hour at room temperature. The membrane was then incubated with primary antibodies overnight at 4°C. After the PBS wash, it was further incubated with HRP-conjugated secondary antibodies at room temperature for 3 hours and was followed by a PBS wash. The blot was developed using a chemiluminescent substrate (Super Signal West Pico, Thermo Scientific - 34080). The intensity of the bands was quantified using the Image J software. Primary antibodies used were HspB5: 1:1500, Enzo life sciences - ADI-SPA-223; Procaspase-3: 1:1500, Cell Signaling Technology - 9915S; β-actin: 1:1500, Abcam - ab8226. Secondary antibodies - anti-rabbit/mouse: 1:3000, Invitrogen /626520.

Statistical analysis: The densitometric measurements of all the detected proteins were performed by Scion NIH Image Analysis Software (Image J) (version 3.5). All western blots were represented as a mean of at least 3 independent experiments. Data are expressed as mean ± SD. Paired 2-tailed Student's t-test was used for the analysis of the significance of the variations. p<0.001 was considered as statistically significant. GraphPad Prism 3 (GraphPad Software, Inc.) was used for statistical analysis.

Results

Protein purification

The recombinant human HspB5 was expressed in *E.coli* and purified to its homogeneity (**Figure 1A**).

Biophysical characterization

Circular dichroism: The far-UV CD spectral data obtained for the recombinant human HspB5

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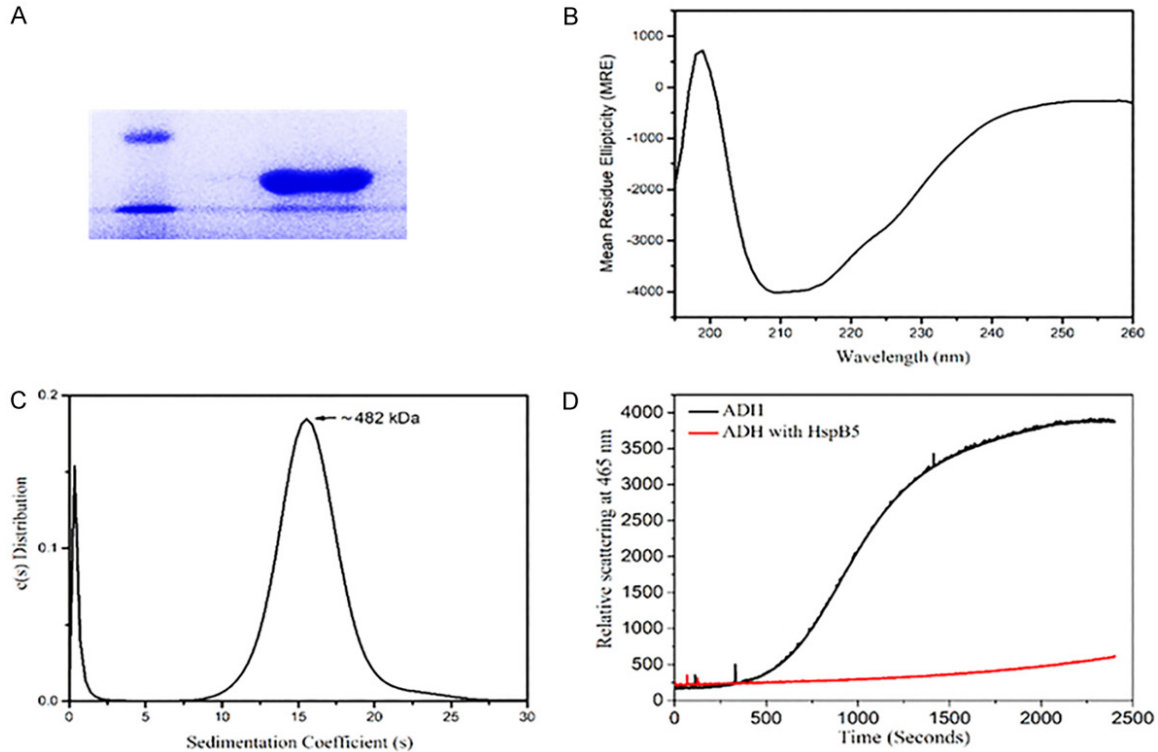


Figure 1. Characterization of the HspB5 protein. A. A 12 % SDS-PAGE shows the purified human recombinant HspB5 at ~20 kDa (lane to the right) against a Low Mol.Wt. protein marker (lane to the left); B. Far-UV CD Spectrum indicates a predominantly beta-sheet structure (minima ~215 nm) along with a slight alpha-helical component, typical of HspB5; C. Sedimentation profile of HspB5 shows a sedimentation coefficient of 16.6 S and a molecular mass of 482 kDa, indicating a 24-mer oligomeric structure; D. HspB5 (red line) shows protection against the temperature-induced aggregation of Alcohol dehydrogenase (black line).

Table 1. Secondary structural studies using Far-UV CD: CDNN analysis of the data suggests that the recombinant HspB5 has beta-sheet structures predominantly

Wave length	Helix	Antiparallel	Parallel	Beta-turn	Random coil	Total
195 nm-260 nm	17.6%	25.0%	14.3%	20.5%	44.1%	121.5%

(**Figure 1B**) was analyzed using the CDNN software (**Table 1**). The protein was found to be predominantly beta-sheet in nature, as reported in earlier studies [31, 32] (**Figure 1B**).

Sedimentation-velocity measurements: The sedimentation coefficient profile of HspB5 showed a peak at 16.6 S (**Figure 1C**). Using the 'SEDFIT' software, the molecular weight of HspB5 was calculated to be 481820 Da or ~482 kDa, indicating that the protein exists as a 24-mer, as reported in the literature [33] (**Figure 1C**).

Chaperone assay: Even at an exceptionally low concentration, 1:0.0625 (ADH: HspB5), HspB5 showed significant protection by preventing the

thermal aggregation of Alcohol dehydrogenase (**Figure 1D**).

Isolation and maintenance of *m*NSPCs

*m*NSPCs isolated from the embryonic day 14 (E14) embryos were cultured and maintained in neural stem cell maintenance media. Immunostaining results showed specific neural stem cell markers (SOX2 and Nestin) (**Figure 2A**). Upon incubation with varying concentrations of FITC-tagged HspB5, *m*NSPCs showed the localization of HspB5 in the cytoplasm and perinuclear region of the cell, showing the capability of protein to cross the cell membrane (**Figure 2B**). Western blot was performed to determine the localization of exogenously

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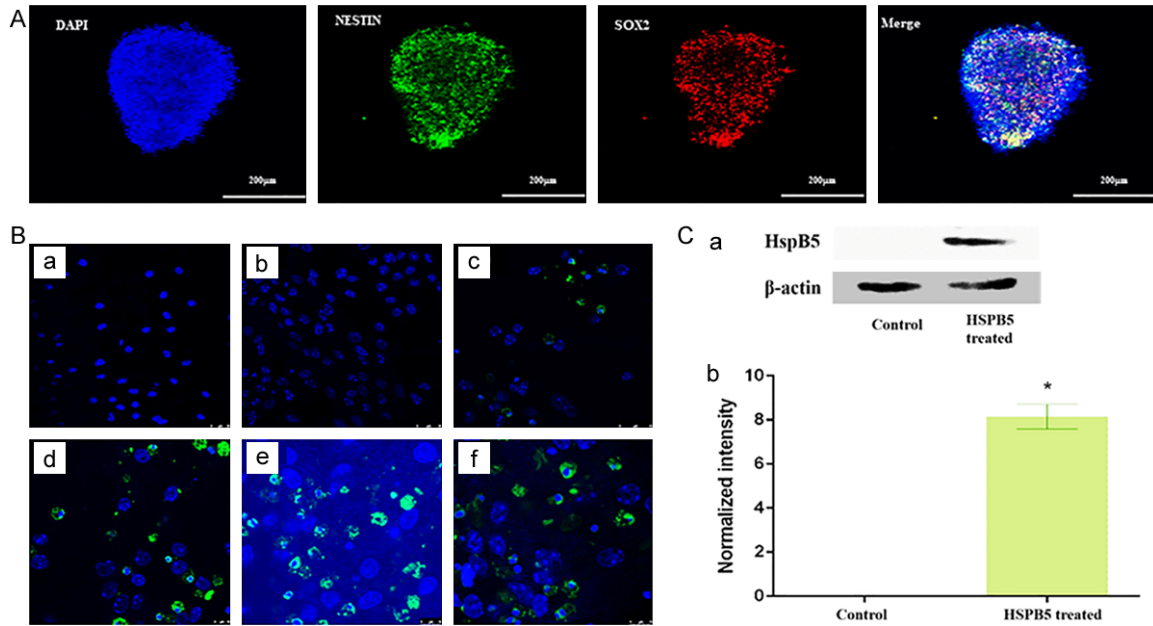


Figure 2. A. Stem cells markers, suggesting the stemness in the cultured Neurospheres; Blue represents Nucleus (DAPI); Green represents Nestin; Red represents SOX2. B. Localization of exogenously added HspB5 (varying concentrations) in *m*NSPCs; (a) Media only (b) 10 μg/mL (c) 50 μg/mL (d) 100 μg/mL (e) 150 μg/mL (f) 200 μg/mL. C. (a) HspB5 Immunoblot. Photograph showing HspB5 expression in treated and untreated groups. (b) The densitometric measurements of all the detected proteins were performed by Scion NIH Image Analysis Software (Image J) (version 3.5). Each measurement represents the mean ± SD of the intensity of the bands normalized to their respective control (β-actin).

added HspB5 in the *m*NSPCs. The presence of HspB5 in the cell lysate for the protein-treated-group, compared to the untreated group, suggests that the externally added protein crossed the cell membrane and localized in the cytoplasm of *m*NSPCs (Figure 2C).

Anti-apoptotic activity of HspB5 against paraquat-induced oxidative stress

Hoechst-PI staining: Hoechst-PI staining was used to distinguish the apoptotic cells from viable cells; a greater proportion of PI-positive cells (red) that represent dead cells were seen in the control *m*NSPCs treated with paraquat. However, the HspB5-treated *m*NSPCs were significantly protected from cell death caused due to paraquat, as evidenced by a decrease in PI-positive cells (Figure 3A).

MTT assay: The metabolic activity of *m*NSPCs after 4 hours of paraquat treatment was assessed by MTT assay. The cell viability between the control and the experimental groups (PQ²⁺, HspB5-PQ²⁺) and between the experimental groups showed significant variation ($p < 0.001$), indicating that HspB5 pre-treatment of *m*NSPCs

confers protection against paraquat-induced-oxidative stress (Figure 3B).

Western blotting: Caspase-3 is known to be produced in a cell as a low activity proenzyme that is cleaved under apoptotic conditions to produce the active enzyme caspase-3 that functions as an 'executioner' caspase. Once activated, it cleaves several cellular substrates committing the cell to apoptotic death [30, 34]. Western blot was performed to determine the localization of exogenously added HspB5 in the *m*NSPCs. The abundance of HspB5 in the cell lysate for the protein-treated-group, compared to the untreated group, suggests that the externally added protein crossed the cell membrane and localized in the cytoplasm of *m*NSPCs. Western blot analysis was performed to assess the involvement of apoptotic proteins in paraquat-induced cell injury. Paraquat treatment decreased the expression of procaspase-3 in the *m*NSPCs, whereas procaspase-3 expression of the *m*NSPCs pre-treated with HspB5 was comparable to that of the control group. In untreated *m*NSPCs, the expression of apoptotic protein procaspase-3 decreased significantly ($p < 0.001$), suggesting the role of HspB5 in pre-

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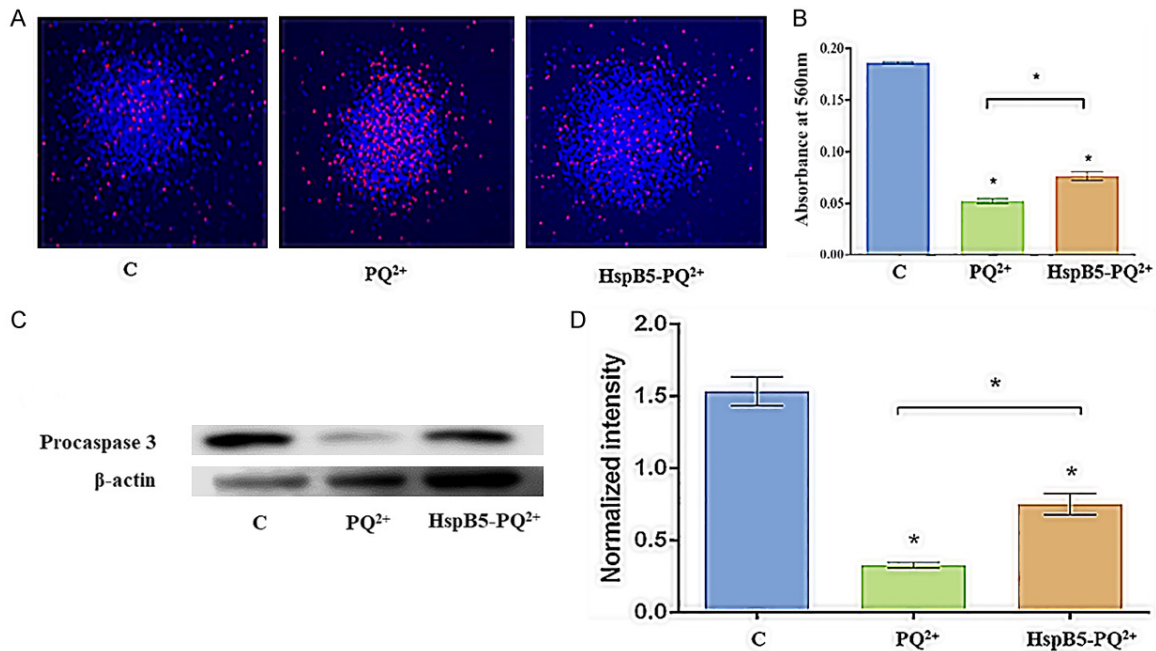


Figure 3. Anti-apoptotic activity of HspB5 against paraquat-induced oxidative stress. A. *m*NSPCs were exposed to 3 mM paraquat for 4 h, stained with Hoechst (10 μ g/mL) - PI (50 μ g/mL) and observed under the fluorescence microscope. Here, [C] represents the control *m*NSPCs without any paraquat stress, [PQ²⁺] represents the *m*NSPCs treated with paraquat, and [HspB5-PQ²⁺] represents the *m*NSPCs pre-treated with HspB5 (100 μ g/mL). Scale bar = 100 μ m; B. Quantification of cell viability using the MTT assay; * represents statistical significance between the different treatment groups at $p < 0.001$. C. Immunoblotting of apoptotic procaspase-3 in *m*NSPCs after paraquat stress. D. Western blot showing the expression of procaspase-3 expression in control, PQ²⁺ treated, and HspB5-PQ²⁺ treated. The densitometric measurements of all the detected proteins were performed by Scion NIH Image Analysis Software (Image J) (version 3.5). Each measurement represents the mean \pm SD of the intensity of the bands normalized to their respective control (β -actin). A significant difference ($p < 0.001$) was found between the different groups by paired 2-tailed Student's t-test.

venting apoptotic cell death during paraquat injury (Figure 3C, 3D).

Discussion

The ability of HspB5 to prevent protein aggregation and its anti-apoptotic role can have therapeutic implications. For example, intravenously injected HspB5 promoted axonal regeneration of the optic nerve in rats after optic nerve crush [35]. Exogenously administered HspB5 rescued the optic nerve oligodendrocytes in an experimental model of anterior ischemic optic neuropathy in mice by inhibiting microglial activation [36]. Because of the advantages of HspB5 in cell-protection from apoptosis, attempts are being made to deliver HspB5 into the cells tagged with a cell penetration peptide (CPP) [26]. The cell-entry strategy of HspB5 into lens epithelial cells showed enhanced protection against various stresses such as heat and oxidative stress [27]. Our studies suggest that

HspB5 can be delivered into *m*NSPCs by exogenously adding the protein to the medium (Figure 2B). The mechanism of uptake is still not clear, and further experiments need to be carried out to understand how the cells take up the exogenously added HspB5 in the medium.

Our studies confirm that 24 h pre-treatment with HspB5 protected *m*NSPCs from paraquat-induced apoptosis. The protective ability of HspB5 was confirmed with a decrease in cell death, as evidenced by Hoechst-PI staining and MTT data (Figure 3A, 3B). The mechanism of protection seems to involve the prevention of activation of procaspase-3 to the mature executioner caspase-3 (Figure 3C, 3D). In agreement with our findings, earlier studies showed that HspB5 interacts with procaspase-3, preventing their maturation to caspase-3 [37, 38]. It is interesting to know that the overexpression of HspB5 in C6 astrogloma cells protects them from H₂O₂-induced apoptosis by inhibiting cas-

pase-3 activation [39]. It is also known to regulate the anti-apoptotic function through caspase-3, Bcl-X, and Bax [40-42] and also known to inhibit the release of cytochrome C from mitochondria by downregulating Bcl-2 in H₂O₂ injured cells [43]. The anti-apoptotic activity of HspB5 has been associated with its translocation to mitochondria, binding with cytochrome c, caspase-3, caspase-12, and voltage-dependent anion channels [44]. The *m*NSPCs take up the FITC-labeled HspB5 upon 24 h treatment, and the protein was found to be localized in the cytoplasm, especially surrounding the nucleus (Figure 2B).

Neurological and neurodegenerative diseases are characterized by neuroinflammation and cell death. HspB5 is demonstrated to have anti-inflammatory activity in many pathological conditions through the inhibition of T-cell proliferation, reduced secretion of pro-inflammatory cytokines, and limiting apoptosis [10]; the anti-inflammatory activity of HspB5 thus reduces the burden of oxidative stress. The secretion of HspB5 via exosomes plays an essential role in suppressing neuroinflammation and can act through autocrine [45] and paracrine manner [46] in suppressing the inflammation in microglial cells.

In the present study, we have demonstrated that exogenously added HspB5 could cross the cell membrane and protect the *m*NSPCs from oxidative stress induced by paraquat. Pre-treatment of the cells with HspB5 decreased the proportion of apoptotic cells when challenged with paraquat. Our study provides compelling evidence that the baseline antioxidant capacity increases with the availability of HspB5, thus leading to stem-cell protection.

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Disclosure of conflict of interest

None.

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