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Postmortem Evaluation of Topoisomerase IIβ, Nurr1, and Neuroinflammatory Signatures in Parkinson's Disease

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Abstract

The selective vulnerability of dopaminergic neurons in the substantia nigra pars compacta is a major mystery in the pathogenesis of Parkinson's disease (PD). A growing body of evidence points to the fact that the neurodegenerative process is a result of a complicated interaction between transcriptional dysregulation and chronic neuroinflammation.

This study specifically investigates three key molecular regulators; DNA topoisomerase IIβ (topo IIβ), Nuclear Receptor Related 1 (Nurr1) and Nuclear Factor kappa B (NF-κB). The hypothesis

posits that the interplay between these factors forms a critical transcriptional-inflammatory axis, and its disruption may significantly contribute to the progression of PD. Using immunohistochemical and western blot analyses on postmortem substantia nigra tissue samples from patients with PD and neurologically healthy controls, the study revealed a pronounced decrease in the expression levels of topo IIβ and Nurr1 in the PD samples.

This decline indicates a substantial deterioration in transcriptional integrity and a departure from the cellular identity of dopaminergic neurons. Concurrentl, the analysis showed a substantial reduction in the unphosphorylated basal forms of the NF-κB p65 subunit, suggesting the potential loss of homeostatic inflammatory signaling, or the loss of the neurons' responsiveness entirely. The finding support a model wherein the failure of primary transcriptional maintenance, indicated by decreased levels of topo IIβ and Nurr1, is the critical for the loss of dopaminergic neuron phenotype and intrinsic mechanisms that uphoald and an anti-inflammatory state within these cells. The deficiency in inflammatory signaling appears to diminish the neurons' resilience against degenerative processes, thereby accelerating neurodegeneration. This research identifies the topo IIβ-Nurr1-NF-κB axis as a novel and upstream pathogenic hub, highlighting its potential as a strategic target for the development of future disease-modifying therapies for PD.

Introduction

Parkinson's disease (PD) is the neurodegenerative disease that accounts for the second greatest prevalence globally. The disease identified through a cluster of clinical motor characteristics (e.g., bradykinesia, rest tremors, rigidity) [1] as an immediate result of a very significant and highly specific loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) [2]. Although the primary pathological event associated with PD is the accumulation of misfolded α-synuclein to form Lewy bodies, recent evidence suggests that early α-synuclein pathology induces neuroinflammation and a corresponding decrease in DNA Topoisomerase IIβ (topo IIβ) expression [3] In line with this, recent studies have shifted the focus from a single causative factor to a convergence of multicellular pathways, identifying transcriptional dysregulation and neuroinflammation as central components of PD pathology [1]

The survival of long-lived, pot-mitotic neurons like those in the SNc depends on the strict, ongoing maintenance of their transcriptional programs. Topo IIβ, encoded by the TOP2B gene is primarily found in terminally differentiated neurons and serves as a critical molecular switch during neural differentiaiton [4], [5] Topo IIβ addresses DNA topological stress by introduction emporary double-strand breaks in the DNA, allowing the transcription of large, activity-dependent genes necessary for neuronal development and survival, specifically for synaptic function [6] . Consequently, the loss of topo IIβ is linked to impaired gene expression and accumulation of DNA damage and neurodevelopmental deficits, making it an important contributor to the structural integrity of the neuron [7], [8] Moreover, the downregulation of topo IIβ has been demonstrated to induce neurodegeneration-like effects via the manipulation of Rho GTPases, particularly by the downregulation of tyrosine hydroxylase (TH) [9]

Neuroinflammation has emerged as a primary mechanism for the destruction of brain tissue in PD [1] with this neuroinflammatory process being driven through the activation of the body's own resident microglial and astrocytic cells [3] . The activation of these cells produces an inflammatory reaction which serves as both a protective response and a destructive one, with chronic or unresolved activation resulting in the release of toxic molecules and pro-inflammatory cytokines that can damage adjacent neurons. Microglia-mediated neuroinflammation can become a chronic, destructive process, where the release of pro-inflammatory cytokines damages adjacent neurons [10] The primary regulator of this inflammatory response is NF-kappa B (NF-κB), which plays a multifaceted role in DA neuron survival. While it can promote apoptosis in disease states, it also serves essential protective functions depending on the cellular environment [11] [12]

The molecular link between transcriptional failure and neuroinflammation may lie in the Nuclear Receptor Related 1 protein (Nurr1), which is also known as NR4A2. Nurr1 is vital for maintaining the DAergic phenotype and suppressing pro-inflammatory genes in glial cells [13] . Nurr1 also functions as an endogenously produced anti-inflammatory agent; it has shown to suppress transcriptional production of pro-inflammatory genes by NF-κB in glial cells through repression. [14] The level of Nurr1 expression is reduced in the substantia nigra compacta (SNc) of PD patients indicating that a lack of this anti-inflammatory agent may lead to the loss of dopamine cell identity and exacerbate the neuro-inflammation present in PD. [13]

This study hypothesizes that a regulatory axis involving Topo IIβ, Nurr1, and NF-κB is necessary for maintaining DA neuron homeostasis, and the first stage of this axis disruption through transcriptional failure serves as the main disease mechanism in PD.

Results

Confirmation of PD Molecular Pathology

To determine the degree of neurodegeneration and to confirm the pathological condition of the PD tissue samples, we assessed the expression levels of NeuN and α-synuclein. NeuN is the most prominent marker for post-mitotic neurons and is employed to evaluate the viability of neurons and determine the amount of cell loss [15]. At the same time, α-synuclein was assessed as the principal protein making Lewy bodies and a certain marker of PD pathology[16] **Fig1** illustrates that the analysis on the comparison between healthy controls and PD tissue has uncovered a severe pathological phenotype in the patient group. It was marked by a major fall in the number of surviving neurons accompanied by widespread deterioration in morphology. Also, the combination of immunohistochemistry and Western blot (WB) analysis revealed a notable reduction in the intensity of the signals of these markers in PD tissues, thus confirming our finding of extensive neuronal loss and protein downregulation connected with the disease state in our samples.

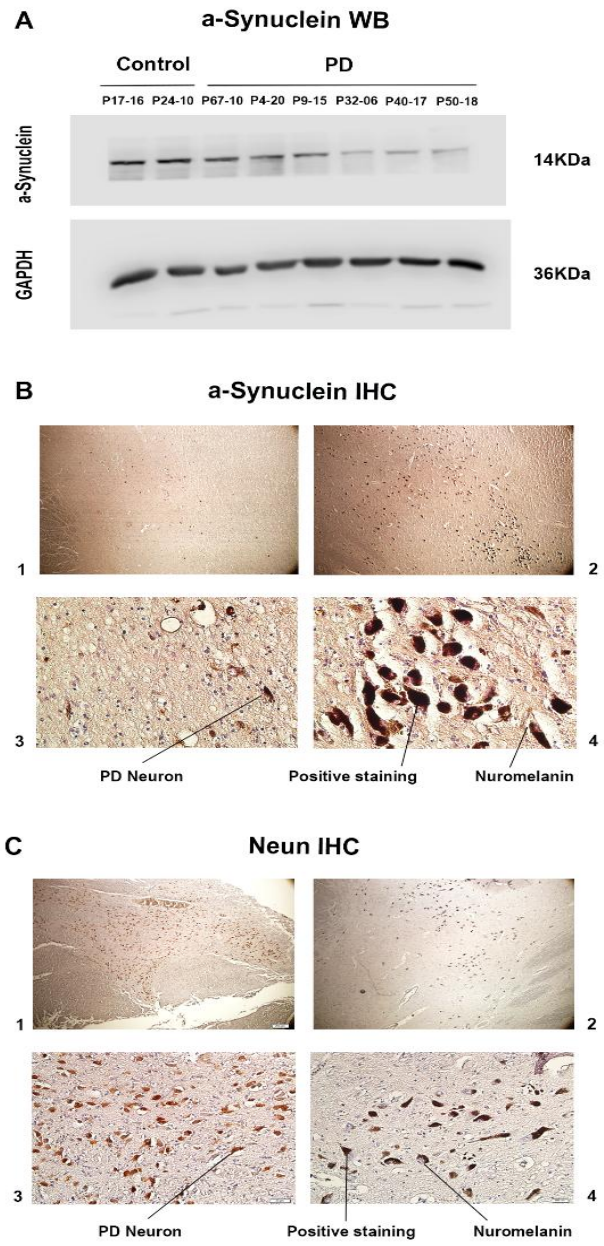


Fig 1 Protein expression levels of NeuN and α-synuclein confirm pathological deterioration in PD. (A) WB of α-synuclein expression in the tissue homogenates indicates that the proteins of interest are downregulated in the PD samples. This is consistent with the immunohistochemical observations regarding the loss of neurons and the decrease in protein expression. (B) The intensity of the α-synuclein signals and the number of cells present were compared, and it was found that there was a significant decrease in the number of neurons as well as a strong reduction of immunoreactivity in the tissues of PD patients ($p < 0.05$). (C) The representative immunohistochemical staining demonstrates NeuN expression in both healthy controls and PD tissue. The PD samples show considerable changes in morphology such as loss of neuronal structural integrity compared to the controls.

Transcriptional Maintenance Machinery is Compromised in the Parkinsonian Substantia Nigra through topo IIβ and Nurr1

The fidelity assessment of transcriptional regulation in PD has been done and demonstrated the protein expression levels of topo IIβ and Nurr1 immunohistochemical (IHC) detection for topo IIβ showed robust and discrete nuclear staining localized in the neurons of the control brains and is consistent with the documented function of topo IIβ as an enzyme in transcriptionally active, post-mitotic neuronal cell bodies. Besides the protein markers, the occurrence of neuromelanin (NM) was considered a very important histological standard for the identification of healthy dopaminergic neurons and the isolation of diseased tissue. Neuromelanin is a dark pigment located mainly in the catecholaminergic neurons of the SNpc and locus caeruleus. As far as its role is concerned, NM is helpful to the neurons because it prevents the entry of toxic reactive iron and environmental toxins, thus, reducing oxidative stress in the neuronal environment [17].

The healthy control subjects showed very clearly the robust neuromelanin pigmentation, which indicated that the dopaminergic population was preserved and functional. On the other hand, the PD tissue samples showed almost complete lack of NM which is indicative of the selective vulnerability and death of pigmented neurons which is a classic feature of PD progression [17]. It is also confirmed by the correlation between the loss of NM and the decreased NeuN signal intensity that the observed neurodegeneration is confined to the specific cell populations that are most impacted by Parkinson's pathology.

On the contrary, topo IIβ staining was significantly reduced in terms of the intensity as well as the quantity of positive staining neurons in the brains of PD subjects. Of the many remaining neurons that were still present with topo IIβ staining there were a large percentage that showed little or no nuclear staining and the majority of those neurons had shrunken nuclei and/or showed other evidence of nuclear disruption (**Fig 2 A**). The quantitative analysis of the stained tissue areas further supported these findings by revealing a statistically significant reduction in the topo IIβ Area Percentage in the PD cohort when compared to control brains ($p < .05$) (**Fig 2 B**). Histologically demonstrated changes were supported at a higher protein level by the use of WB to analyze homogenized SNc tissue from both control and PD groups. Bands with an estimated molecular mass of approximately 180 kDa, corresponding to the predicted size of topo IIβ, were visible in all control tissues examined. However, the intensity of this band was consistently

decreased in tissues analyzed from PD patients compared to those from controls. These results were further supported by densitometry analysis comparing GAPDH protein as a reference to measure topo IIβ protein density in each sample. These measurements demonstrated that PD tissues contained a statistically significant lower amount of topo IIβ than did control tissues ($p<0.05$) (**Fig 2 C-D**).

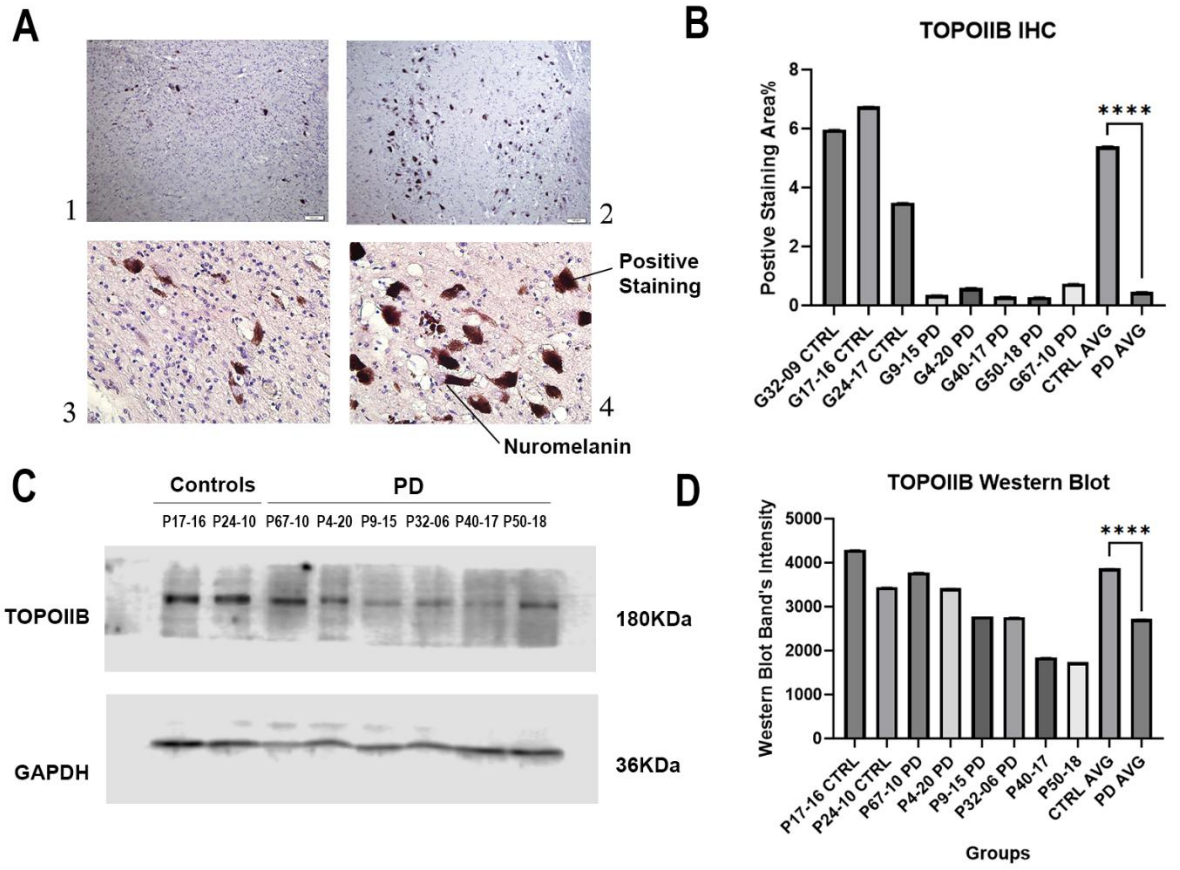


Fig 2 Impairment of topo IIβ in the Parkinsonian Substantia Nigra. (A) The demonstration of topo IIβ through immunohistochemical (IHC) staining in the SNc. Topo IIβ in the tissue of the healthy control, presents strong and distinct nuclear staining inside the post-mitotic neurons, supporting its function in the maintenance of the transcriptionally active DNA. On the other hand, samples from PD patients exhibit a drastic reduction in both signal strength and the number of positive neurons. The remaining neurons in the PD tissue often have small nuclei or complete nuclear destruction. The healthy individuals present considerable amounts of neuromelanin and marked neuronal immunoreactivity, but in the case of PD tissues the pigmented neurons are substantially decreased. (B) A graph of the topo IIβ Area Percentage confirms that there is a significant difference between the PD and healthy control groups, with the PD group having lesser area coverage ($p < 0.05$). (C–D) The analysis of homogenates of SNc tissue using WB. The control samples show a clear band at 180 kDa, which is the expected molecular weight for topo IIβ while the PD lanes show that the intensity is greatly reduced. GAPDH-normalized densitometry agrees with the significant reduction of topo IIβ protein levels in the PD group ($p < 0.05$).

There was a parallel loss of Nurr1 as well, this is the major transcription factor responsible for DA neuronal identity. IHC staining in normal tissue samples had strong Nurr1 localization to SNc neuronal nuclei. In contrast, IHC staining demonstrated a marked decrease in the number of PD SNc neurons staining for Nurr1, as well as a marked decrease in the intensity of staining in PD SNc neurons compared to controls (**Fig 3 A**). Quantitative analysis of Nurr1 area percentage in PD tissues confirmed that Nurr1 Area Percentage was significantly lower than that in controls (P = 0.05) (**Fig 3 B**).

Further validation of these findings was provided by WB analysis of Nurr1. The presence of a clear band at approximately 70 kDa in control lanes is clearly observable. However, the density of this band is dramatically decreased in all PD patient lanes examined (**Fig 3 C).** Quantitation of the overall level of Nurr1 protein in PD patient tissue relative to control tissue confirmed a statistically significant reduction in the level of Nurr1 protein ($p < .05$) (**Fig 3 D**).

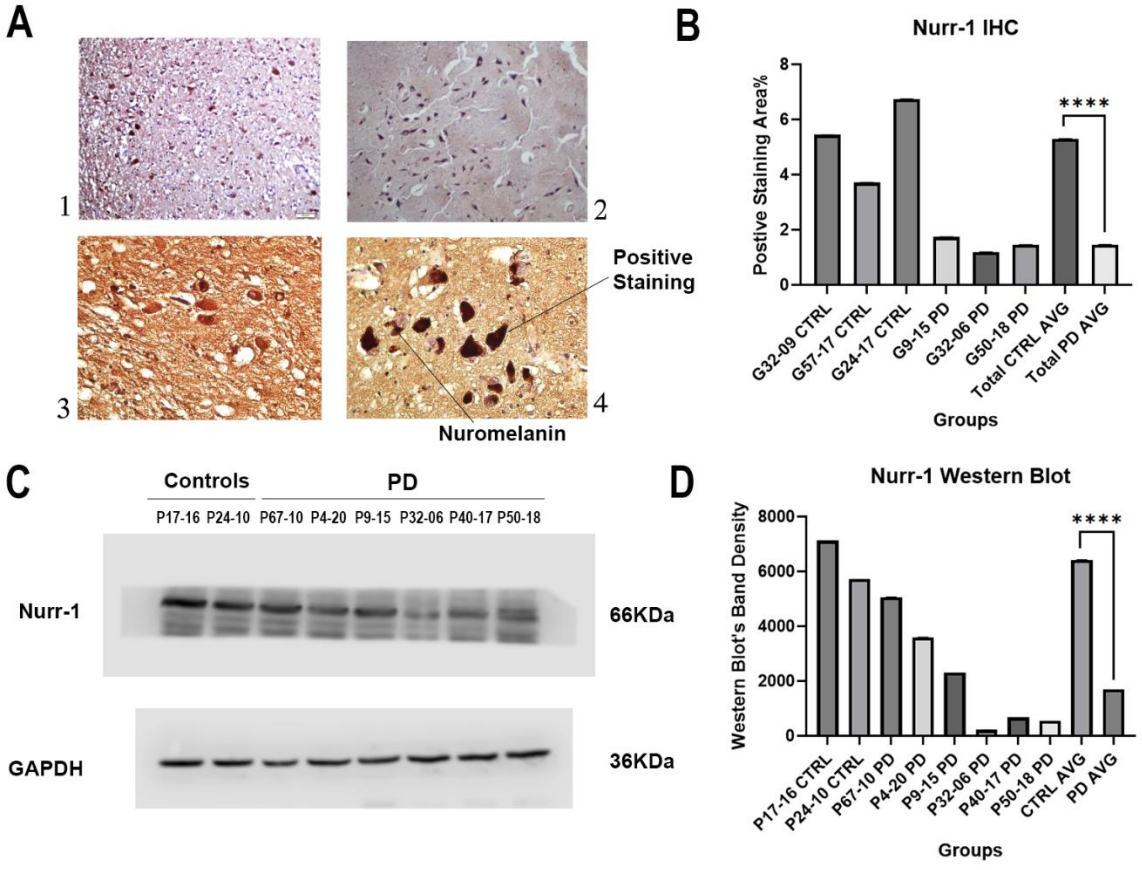


Fig 3 Downregulation of the Dopaminergic Transcription Factor Nurr1**.** (A) In the SNc, IHC staining was done for Nurr1. Control tissues exhibited a high concentration of Nurr1 closely confined to neuronal nuclei which is a prerequisite for the maintenance of the dopaminergic phenotype. PD tissues showed a considerable decrease in the total number of Nurr1-positive neurons and also a substantial reduction of the staining signal. (B) The quantitative evaluation of Nurr1 Area Percentage confirms the significant reduction of this transcription factor in PD patients ($p = 0.05$). (C–D) Nurr1 protein levels were verified by western blotting. The distinct 70 kDa band is very clear in the control group but is either very low or non-existent in the PD patient group lanes. The quantitative densitometry study shows a statistically significant decrease in the total Nurr1 protein in the PD group when compared to healthy controls ($p < 0.05$).

These data collectively indicate that the levels of two proteins, which are crucial in the maintenance of gene expression within neurons and specific dopaminergic cell types, are profoundly reduced in the SNc of PD patients. These data therefore suggest an extensive and profound disruption of normal transcriptional regulation that may be central to the pathophysiology of PD.

Evidence of Dysregulated Inflammatory Homeostasis in PD

The activation status of the central inflammatory mediator NF-kappa B was assessed by determining the expression levels of its total (i.e., predominantly unphosphorylated) p65 subunit. In control tissue, using IHC, NF-kappa B showed moderate staining that was primarily cytoplasmic in the SNc neurons, indicating a basal level of expression of NF-kappa B in normal cells. The staining was clearly less intense and more scattered in PD tissue compared to controls and there was a clear overall loss of immunoreactive signal (**Fig 4A**). A quantitative measure of the intensity of the IHC staining, i.e., NF-kappa B Area Percent, was significantly reduced ($p < 0.05$) in the PD group as shown in Fig 4B.

The decrease in total protein also demonstrated through WB, as there were bands present for all samples that corresponded to the NF-κB p65 subunit at approximately 65 kDa. However these bands generally were less intense than those from control samples (**Fig 4C**). There was an average of a statistically significant decrease in total NF-κB p65 protein ($P = .05$) relative to GAPDH across all samples from the PD group (**Fig 4D**).

The data collected indicate that there is less total number of the major inflammatory transcription factor (NF-κB) to be utilized by the inflamed tissue of the PD substantia nigra as opposed to simply an increase in amount due to chronic inflammation. Therefore, either a significant decrease in the number of neurons which express this factor has occurred, or, alternatively, a fundamental

alteration in the regulatory (homeostatic) mechanism for inflammatory signaling has taken place within the remaining tissue.

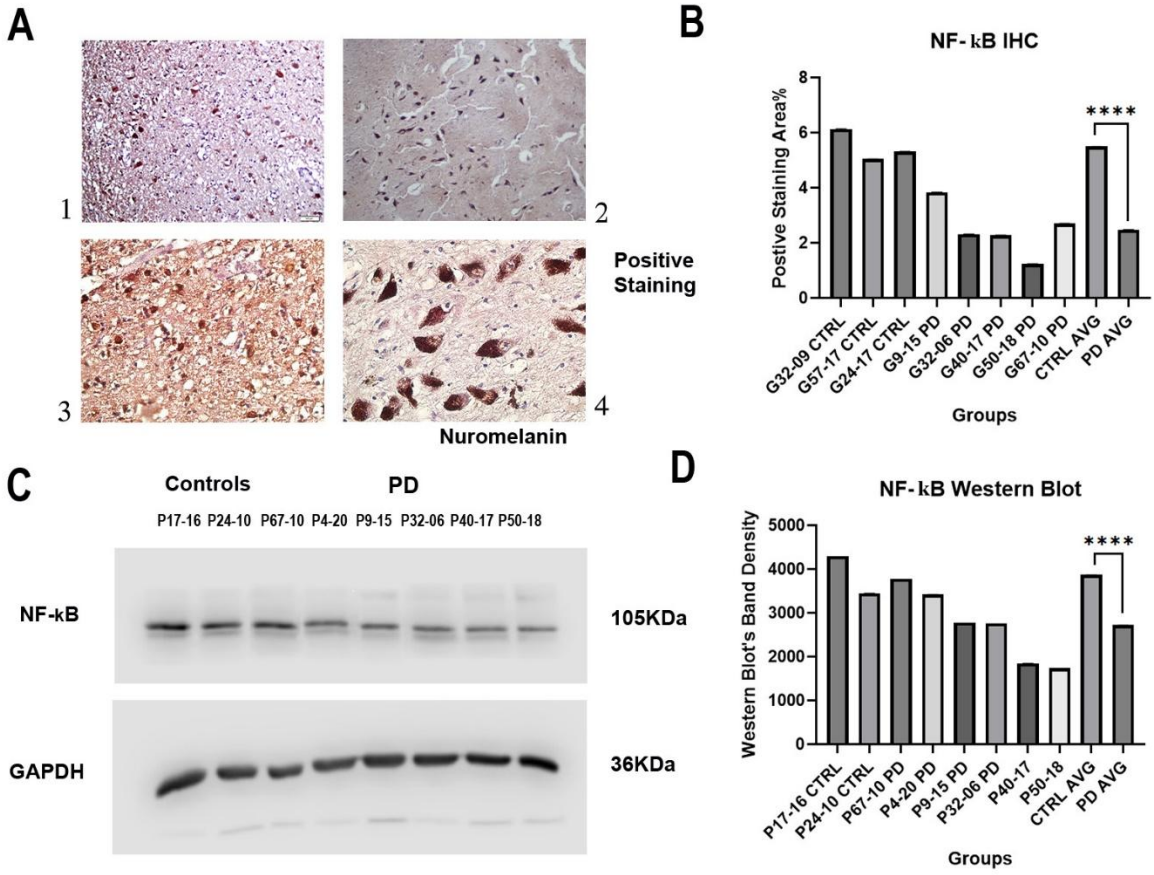


Fig 4 Reduction of total NF-κB p65 subunit expression in the PD substantia nigra. (A) Representative IHC staining of the NF- κB p65 subunit in the SNc. In the case of healthy control tissues, p65 reveals moderate and mainly cytoplasmic staining which denotes basal homeostatic expression. The immunoreactive signal in PD tissues is significantly less intense, dispersed, and demonstrates an overall loss of staining density. (B) NF- κB Area Percentage quantitative analysis, revealing a statistically significant decline in p65 expression in the PD group when compared to healthy controls ($p < 0.05$). (C-D) Western blotting and densitometry for total NF- κB p65. Protein bands at around 65 kDa are seen in all samples, however, the intensity of the band is greatly reduced in PD samples. Normalization to GAPDH shows a statistically significant reduction in total p65 protein levels in the PD group ($p = 0.05$).

Discussion

The data from this study provide significant evidence that topo IIβ, Nurr1, and basal NF-kappa B are simultaneously down regulated in the SNc of PD patients.

These should be viewed as interrelated components of a failing regulatory axis rather than isolated events. Interestingly, this pattern of Topo IIβ and Nurr1 decrease is also observed in cellular models of Alzheimer's disease (AD) induced by Aβ1-42 peptides, suggesting a common theme of transcriptional failure across neurodegenerative pathologies [18]

A Unified Model of Transcriptional Collapse and Inflammatory Dysfunction

One of main outcome of this research was that there is a decrease in the overall or unphosphorylated NF-κB p65 subunit pool. In PD, the chronic neuroinflammatory state has been related to the activation of NF-κB by phosphorylation and nuclear translocation in glia which leads to pro-inflammatory gene expression. However, the transcription factor NF-κB plays important basal protective neuronal functions as well [11]

The basal NF-kappa B activity enhances the production of anti-apoptotic proteins (i.e., Bcl-2, IAPs) and of anti-oxidant enzymes (e.g., Mn-SOD) necessary for neuron survival when under stress conditions [12]. Thus the diminished total protein likely represents a complete lack of the neuroprotective capability that the lost cells had, with the remaining cells being unable to generate a sufficient homeostatic response to the increasing number of cellular insults of PD (e.g., oxidative stress, mitochondrial dysfunction) [19]. In this respect, the loss of these protective mechanisms is more significant than simply chronic activation; it is the loss of an important cell defense mechanism.

This transcriptional collapse is heavily tied to the massive reduction of topo IIβ. SNc DA neurons are among the most metabolically demanding cells in the body, possessing complex, long axons that require immense transcriptional activity to maintain function [6] Topo IIβ facilitates the transcription of the long genes necessary for these functions by relieving mechanical strain genes [20]. Beyond structural maintenance, topo IIβ has been shown to stimulate neurite outgrowth through the regulation of Rho-GTPases (specifically the RhoA/Rock2 pathway) and Nurr1 expression Therefore, its loss leads to transcriptional stress and a failure to maintain neuronal viability [21]. Therefore, this transcriptional failure could provide a reasonable mechanism for widespread cellular dysfunction.

Due to its role in forming the link between transcriptional and inflammatory mechanisms, Nurr1 may be an essential component in the proposed pathogenetic mechanism. It is also possible that Nurr1 will be reduced due to a general failure of transcription or as a result of a direct reduction of certain downstream pathologies. The reduction of Nurr1 would have two simultaneous effects. First, Nurr1 would reduce the number of genes that define a dopaminergic neuron. Second, Nurr1 would eliminate the major endogenous negative regulatory control on glial inflammation. Nurr1 has been shown to inhibit NF-κB activity in microglia and astrocytes, thus inhibiting the expression of neurotoxins produced through the inflammatory process [13]**.** Therefore, the depletion of Nurr1 in the SNc microenvironment eliminates the neuron's intrinsic anti-inflammatory mechanism of action and places the neuron and glial cells that are in contact with it into a condition where they can produce a dysfunctional inflammatory reaction (**Fig 5**).

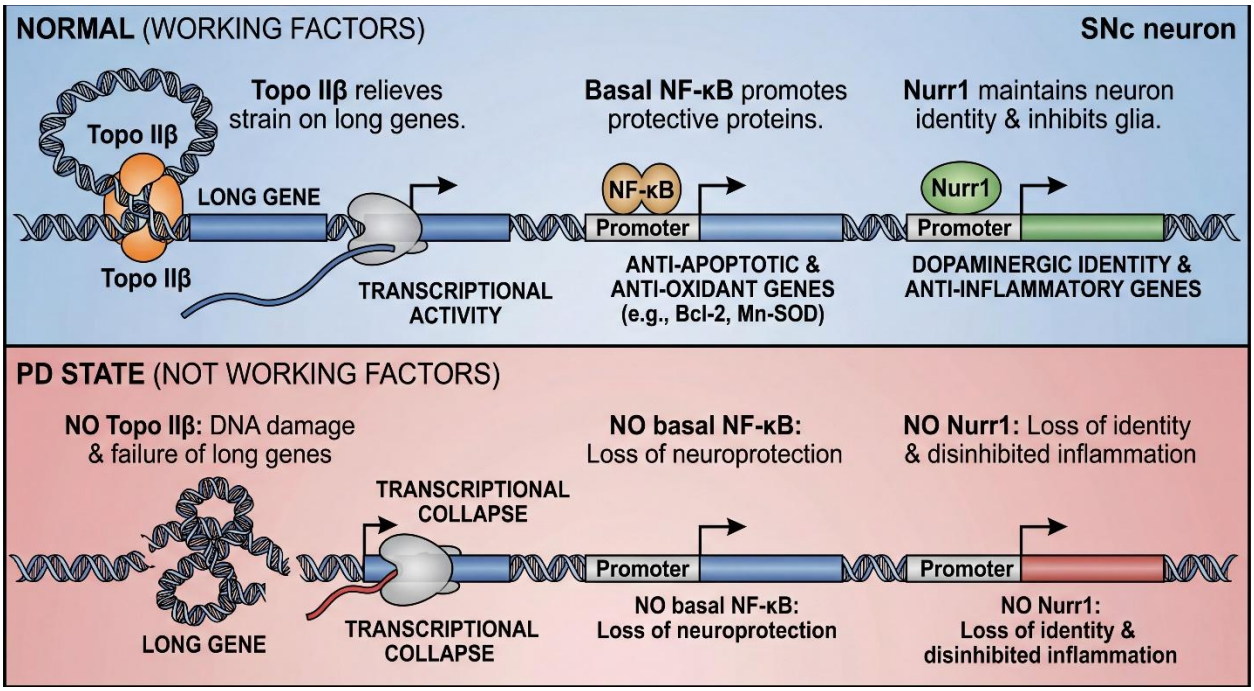


Fig 5 Hypothetical figure demonstrating the topo IIβ-Nurr1-NF-κB axis and selective vulnerability. The schematic demonstrates that three particular transcription factors must remain present to protect neurons from developing dysfunction. First, topo IIβ deficiency prevents SNc neuron from expressing essential long genes required for its metabolic activities. Second, the cell loses its ability to create protective proteins against oxidative stress and cell death through the absence of basic NF-κB function which includes Mn-SOD and Bcl-2 as elements of protection. The Nurr1 decrease establishes a connection between transcriptional breakdowns and inflammatory process disturbances because it eliminates the natural NF-κB activity restraint which existed in adjacent glial cells. The combined loss of these molecules creates a unified model of cell-intrinsic transcriptional failure driving neurodegeneration.

A Cell-Intrinsic Driver of Neurodegeneration

The results from these studies are supportive of a hypothetical model for how the neurodegenerative cascade occurs in PD that involves the intrinsic failure of the dopaminergic neuron to maintain homeostasis and function properly. The execution of programmed cell death is dependent on many non-cell-autonomous elements such as activation of microglial cells and astrocytes [3]. However, based on the data reported here, it appears that the intracellular topo IIβ-Nurr1-NF-κB axis fails first leading to a neuron that is no longer functional in terms of its unique identity or in terms of its capacity to respond to stressors and inflammatory conditions. As a result, the neuron would likely produce DAMPs and misfolded α-synuclein, which would then stimulate the surrounding glia (microglia and astrocytes) [22]. The stimulation of glia leads to an activation of the glia, resulting in the release of additional factors that can lead to further damage to the neurons, creating a "vicious cycle" of neuroinflammation [23]. Thus, this hypothetical model identifies transcriptional failure as being the initial and most significant factor in the development of the degenerative cascade in PD (**Fig 6**).

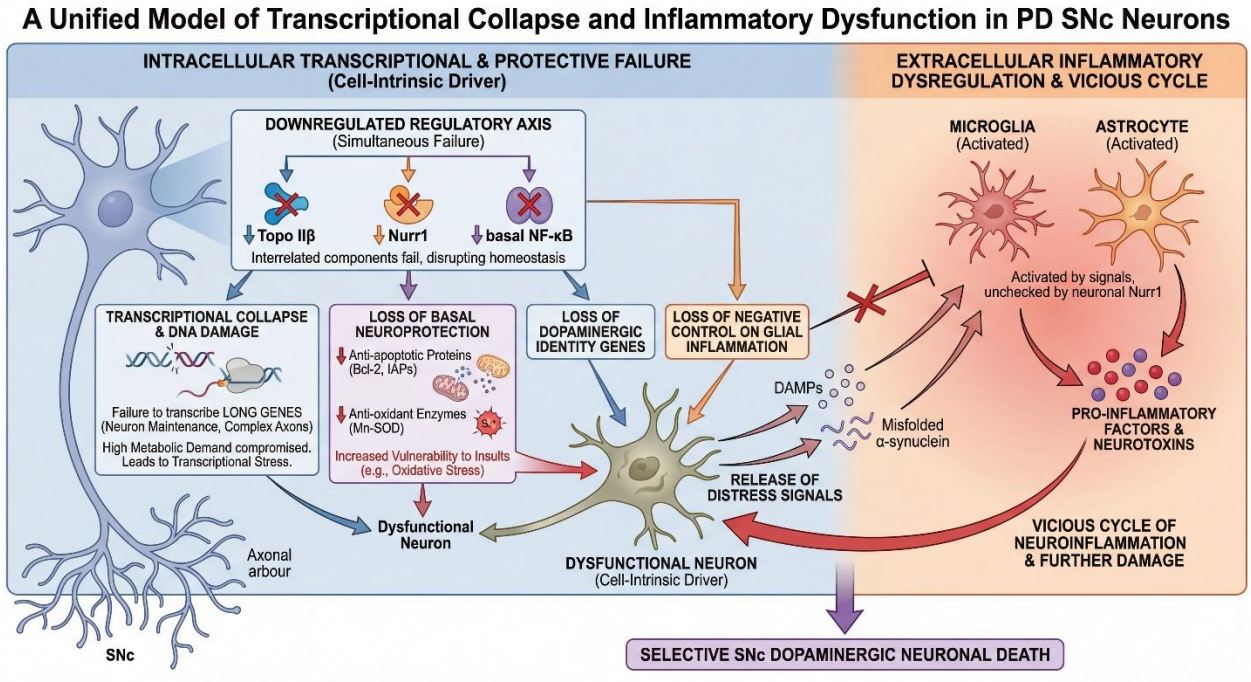


Fig 6 An Hypothetical Model From Transcriptional Collapse to Neuroinflammation. A model of the pathogenic cascade in the Substantia Nigra. The transcriptional collapse starts when a cell loses its ability to process biological information through the simultaneous reduction of Topo IIβ and Nurr1 and the basal NF-κB p65 subunit. The neuron becomes incapable of dealing with high energy needs and protecting itself from oxidative damage because of this internal malfunction. The

dying neuron releases inflammatory signals DAMPs and misfolded α-synuclein which activate adjacent glial cells. The Nurr1-mediated brake on inflammation results in uncontrolled glial activation which creates a loop of neurotoxicity that leads to ongoing neuronal death.

Therapeutic Implications

Identifying this axis provides a new target for disease-modifying therapies. The current treatment options which exist for PD treat only its symptoms while the therapeutic approach that uses small molecule Nurr1 and topo IIβ agonists to restore the topo IIβ-Nurr1-NF-κB axis function will enable restoration of the DAergic phenotype together with the neuron's natural ability to fight inflammation. [13]. Future research using iPSC models and human brain organoids will be essential to determine the chronological sequence of these events, specifically whether topo IIβ loss consistently precedes Nurr1 downregulation. Additionally, ChIP-seq analysis can clarify how Topo IIβ and Nurr1 binding to specific gene promoters is altered in PD.

Limitations and Future Directions

This research relies on post-mortem human brain tissue, which provides a static, terminal snapshot of PD and prevents causative or temporal conclusions. The limited sample size restricts generalizability and heightens vulnerability to inter-individual variability. Consequently, validation in larger, independent cohorts is necessary.

Subsequent research utilizing patient-derived iPSC dopaminergic neurons may elucidate the chronological sequence and molecular interrelations among topo IIβ, Nurr1, and NF-κB dysregulation throughout disease progression. Human brain organoid models provide a physiologically realistic platform to more accurately replicate the biology of the SNc, including neuromelanin synthesis.

Additional examination of neuroinflammatory signaling, encompassing phosphorylated NF-κB activation and cell-type-specific nuclear translocation, is necessary. Simultaneously, ChIP-seq methodologies could clarify whether modified topo IIβ and/or Nurr1 promoter binding contributes to transcriptional dysregulation in PD.

Conclusion

This study provides evidence for a significant and coordinated downregulation of topo IIβ, Nurr1, and basal NF-κB in the postmortem substantia nigra of PD patients. These findings implicate a compromised transcriptional-inflammatory regulatory axis as a central element in the progressive degeneration of dopaminergic neurons. The observed decreases in topo IIβ and Nurr1 point to a fundamental failure in maintaining genomic stability and neuronal identity, while the altered NF-κB expression suggests a crippled homeostatic signaling capacity, leaving neurons vulnerable to cellular stress and damage. Understanding this transcription-to-inflammation pathway opens new avenues for therapeutic strategies that aim to restore this molecular balance, offering the potential to slow or prevent neurodegeneration in PD rather than merely treating its symptoms.

Methods

Human Tissue Samples

Human post-mortem substantia nigra tissue was obtained from The Queen Square Brain Bank for Neurological Disorders, part of UCL Institute of Neurology. Tissue was supplied as both formalin-fixed-paraffin embedded (FFPE) tissue block samples and frozen tissue samples. Ethical approvals were granted by Institutional Review Boards at Uskudar University and The Queen Square Brain Bank and all work was conducted in compliance with institutional policies and practices.

The FFPE tissue samples were from a total of ten subjects; four neurologically healthy control subjects (P24/10, P17/16, P32/09, P57/18), and six subjects with PD (PD) (P67/10, P32/06, P40/17, P9/15, P50/18, P4/20). The frozen tissue samples were also from a total of eight subjects; two neurologically healthy control subjects (P17/16, P24/10); and six subjects with PD (P4/20, P67/10, P9/15, P50/18, P32/06, P40/17)

Immunohistochemistry (IHC)

Formalin-fixed paraffin-embedded (FFPE) tissue blocks were sliced to a thickness of 10 μm and affixed on charged glass slides. Sections were subjected to heating at 60 °C for 2 hours to promote deparaffinization, subsequently immersed in xylene for 30 minutes, and rehydrated using a graded ethanol series. Endogenous peroxidase activity was inhibited by incubation with 3% hydrogen

peroxide in methanol. Antigen retrieval was conducted by boiling the sections in a 10 mM EDTA buffer (pH 8.0) in a microwave oven at 800 W for 30 seconds, followed by incubation at 200 W for an additional 20 minutes. The slides were subsequently permitted to cool at room temperature for 20 minutes. Following a wash with phosphate-buffered saline (PBS), non-specific binding sites were inhibited using Super Block Solution (Sensitek HRP Anti-Polyvalent Lab Pack) for 30 minutes. Sections were subsequently treated with primary antibodies diluted in antibody solution at the following concentrations: Nur1 (1:50), topoisomerase IIβ (1:100), NeuN (1:400), NF-κB p65 (1:400), and α-synuclein (1:100). Incubation occurred for 24 hours at 4 °C within a humidified chamber. On the subsequent day, slides were rinsed in PBS and treated with Sensitek Anti-Polyvalent Biotinylated Secondary Antibody for 30 minutes, followed by a 30-minute incubation with Sensitek HRP reagent. Immunoreactivity was detected utilizing a 3,3′-diaminobenzidine (DAB) chromogen kit. Sections were counterstained with Mayer's hematoxylin, dehydrated through a graded series of ethanol, treated with xylene, and subsequently mounted with Entellan mounting media.

Western Blotting

Homogenization of approximately 10 mg of frozen SNc tissue occurred via a mechanical homogenizer equipped with steel beads in 5 ml RIPA buffer which was further supplemented with 5% sodium dodecyl sulfate (SDS). Total protein concentrations were calculated using the BCA Protein Assay Kit (Thermo, A53226), and 65 ug of total protein per sample was separated by SDS PAGE. The proteins were subsequently transferred to a PVDF membrane. Membranes were blocked for 1 hr at RT with Tris-Buffered Saline with 0.1% Tween-20 (TTBS) that included 5% nonfat skim milk. Membranes were next incubated at 4°C for 24 hrs with primary antibodies diluted as following (anti-topo IIβ 1:1000, Nurr1 1:1000, NF-κB p65 (D14E12) 1:1000, GAPDH 1:7500) that had been diluted into the blocking solution. Membranes were washed three times with TTBS after which they were incubated for one hour at room temperature with an HRP conjugated secondary antibody (GAM IgG, HRP-linked 1:5000, GAR IgG 1:5000) (additional information about antibodies in supplementary) Bands of the proteins were visualized using WesternBright

ECL substrate (Advansta, K-12045-D50), and images of the blots were captured using the LI-COR C-Digit Blot Scanner.

Quantitative and Statistical Analysis

IHC images were taken at 4x, 20x, and 40x magnification. For IHC, a quantitative analysis of images was completed by calculating the percentage of area stained in three representative fields per sample. This calculation determined the average percentage of area stained for each treatment group (control vs. PD).

WB bands were quantitated using densitometry software. Band intensity for each target protein was then divided by band intensity for the respective GAPDH band(s) on the same gel to correct for variability in protein load across lanes.

Statistical differences were analyzed between PD and control groups for both IHC and Western blot data using an ANOVA (one-way). Statistical significance was defined as a p value of < 0.05.

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Author Contributions

M.T. was responsible for the design of the experiments, their execution, the data analysis, and writing the manuscript. S.I. was the one who generated the idea for this research, managed the project, obtained the funding, and was involved in the editing of the manuscript. D.A. was the one who gave the guidance and was in charge of IHC experiments of the project. B.Y.K. guided and supervised the WB experiments. All authors have agreed upon the content of the final manuscript after having read it.

Declarations

Ethical approval This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. This research received its approval from Uskudar University ethics committee for non-interventional research (ISSUE:61351342-September 2023-20) as well as Confirmation by the HTA Person Designated (PD) for the Queen Square Brain Bank for

Neurological Disorders at the 1, Wakefield Street Satellite Site under the UCL Institute of Neurology HTA License Number 12198 (Research Sector) that the research project has been approved by this tissue bank's MTA Approval Committee.

Human Ethics and Consent to Participate Human postmortem tissue samples were obtained from Queen Square Brain Bank for Neurological Disorders at Written informed consent for research use was obtained from the donors or their legal next of kin at the time of tissue donation, in accordance with institutional and national ethical guidelines. The authors did not have direct contact with the donors.

Competing interests The authors declare no competing interests.

Data Availability No datasets were generated or analyzed during the current study.

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Clinical trial number not applicable

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