Movement Disorders
CLINICAL PRACTICE
RESEARCH ARTICLE

The Presence of Alpha-Synuclein in Skin from Melanoma and Patients with Parkinson's Disease

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Abstract: **Background:** The misfolding and prion-like propagation of the protein α-synuclein (α-syn) is the leading molecular signature in Parkinson's disease (PD). There is a significant coincidence of PD and melanoma that may suggest a shared pathophysiology. This study compared the presence of α-syn in neural crest-derived tissues, such as nevi, melanoma, skin tags, and skin biopsies from patients with PD and healthy controls.

Methods: Biopsies from participants with PD were obtained from patients from a tertiary referral center for dermatology and neurology in Mexico and a private dermatopathology center in Florida between January 2015 and March 2016. Biopsies from 7 patients with melanoma, 15 with nevi, 9 with skin tags, 8 with PD, and 9 skin biopsies from healthy volunteers were analyzed for immunohistochemical determination of α-syn and tyrosinase. All analyses were performed by pathologists who were blinded with respect to the clinical diagnosis.

Results: In healthy controls, positive α-syn status was restricted to scattered cells in the basal layer of the epidermis and accounted for 1 ± 0.8% of the analyzed area. In patients with PD, there was increased staining for α-syn PD (3.3 ± 2.3%), with a higher percentage of positive cells in nevi (7.7 ± 5.5%) and melanoma (13.6 ± 3.5%). There was no increased staining in skin tags compared with healthy controls.

Conclusion: Patients with PD and melanoma have increased staining for α-syn in their skin. The authors propose that neurons and melanocytes, both derived from neuroectodermal cells, may share protein synthesis and regulation pathways that become dysfunctional in PD and melanoma.

The prevalence of melanoma is higher in patients with Parkinson's disease (PD) than in the general population,[1,2] and a personal or family history of melanoma is associated with an increased risk of PD.[3,4] Melanin, a skin pigment produced by melanocytes and transferred to keratinocytes, provides protection from solar radiation[5] (Fig. 1). This pigment is synthesized from tyrosine within the melanosome through an enzymatic process involving tyrosinase.[6] In the central nervous system (CNS), neuromelanin is a pigment produced by a nonenzymatic process in the dopaminergic cells of the substantia nigra. Neuromelanin shares chemical similarities with melanin,[7] and 1 of its proposed functions is to serve as a depot for toxins, drugs, and metabolites of dopamine.[8] Alpha-synuclein (α-syn), a protein studied within the context of the pathophysiology of PD, is found aggregated in CNS lesions (called Lewy bodies) from patients with PD and displays a neuron-to-neuron propagation.[9] Neuromelanin-containing neurons in the substantia nigra easily degenerate in the presence of augmented α-syn in patients with

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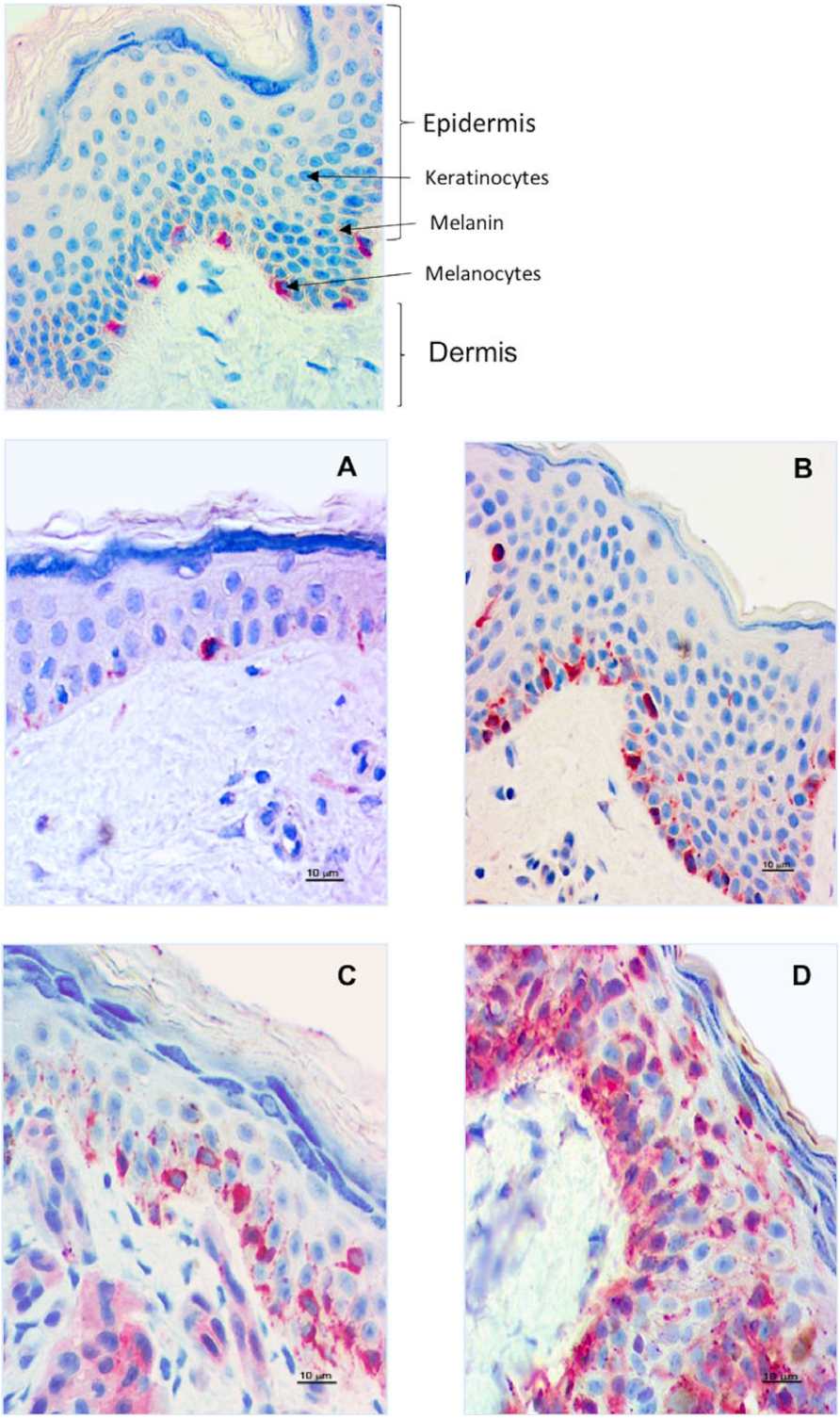

Figure 1 Alpha-synuclein (α-syn) expression in the epidermis. (Top) Details of skin structure show the epidermis, melanin in the cytoplasm of keratinocytes, tyrosinase-positive cells (melanocytes) revealed by immunohistochemistry, and dermis. **A:** Healthy control sample with α-syn immunopositivity in scattered cells of the basal layer of the epidermis. **B:** Parkinson disease sample with multiple α-syn–immunopositive cells in the basal layer, intercellular spaces, and cytoplasm of some keratinocytes. **C:** In a compound nevus, α-syn–positive cells in the dermis and epidermis reach several rows of keratinocytes. **D:** In melanoma, intense immunopositivity is observed along all epidermis strata (amino-ethylcarbazole staining; scale bar = 10 µm).

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TABLE 1 Characteristics of the participants

Case	Age	Sex	Site	Diagnosis
1	51	F	Occipital area	Healthy control
2	77	F	Occipital area	Healthy control
3	63	F	Occipital area	Healthy control
4	64	M	Occipital area	Healthy ontrol
5	58	F	Occipital área	Healthy control
6	69	F	Occipital area	Healthy control
7	54	F	Occipital area	Healthy control
8	77	F	Occipital area	Healthy control
9	76	M	Occipital area	Healthy control
10	22	F	Face	Skin tag
11	33	F	Neck	Skin tag
12	48	F	Face	Skin tag
13	52	F	Face	Skin tag
14	50	F	Face	Skin tag
15	70	F	Face	Skin tag
16	68	F	Face	Skin tag
17	35	M	Abdomen	Skin tag
18	55	F	Armpit	Skin tag
19	70	M	Occipital area	Parkinson's disease
20	68	F	Occipital area	Parkinson's disease
21	55	M	Occipital area	Parkinson's disease
22	61	F	Occipital area	Parkinson's disease
23	36	M	Occipital area	Parkinson's disease
24	73	M	Occipital area	Parkinson's disease
25	62	M	Occipital area	Parkinson's disease
26	64	M	Occipital area	Parkinson's disease
27	33	M	Scalp	Intradermal nevus
28	29	M	Scalp	Junctional dysplastic nevus
29	64	F	Left buttock	Junctional dysplastic nevus
30	40	F	Right forearm	Compound dysplastic nevus
31	51	M	Left shoulder	Compound dysplastic nevus
32	3	M	Nose	Spitz nevus
33	1	M	Scalp	Spitz nevus
34	26	M	Right flank	Spitz nevus
35	8	F	Right buttock	Spitz nevus
36	1	M	Right knee	Spitz nevus
37	17	M	Left clavicle	Spitz nevus
38	12	M	Left elbow	Spitz nevus
39	15	M	Left back	Spitz nevus
40	16	M	Right mid back	Spitz nevus
41	2	M	Left thigh	Spitz nevus
42	62	F	Left upper back	Melanoma in situ
43	86	M	Left forearm	Melanoma
44	89	F	Right leg	Melanoma
45	66	M	Mid sternum	Melanoma
46	81	M	Right back	Melanoma
47	89	M	Left arm	Melanoma
48	34	F	Chest	Atypical melanocytic neoplasm

PD.[10] In addition, α-syn is not exclusive to the CNS but, rather, is a ubiquitous protein found in higher quantities in melanoma. These facts suggest that certain factors related to melanin and α-syn may influence both diseases,[11] namely, perhaps oxidative stress/inflammation, autophagic dysfunction, and proteic aggregation.[12]

A significant finding was the higher expression of α-syn in epidermis from patients who had PD compared with healthy controls.[13,14] Because this protein becomes misfolded in the brain and its aggregates propagate within neurons,[15,16] we hypothesized that the same phenomenon could happen in the skin, as this protein could originate from cells of neural crest origin, like melanocytes, Merkel cells, and nevus cells.

The α-syn protein is also expressed in skin biopsies from patients with melanoma and in nevi tissues.[11] The increased presence of α-syn suggests that this protein could be related to the higher incidence of melanoma in patients with PD versus the general population. Exposure to some pesticides that disturb either mitochondrial energy production or dopaminergic systems are a significant shared environmental risk for PD and melanoma.[17] In addition, in vitro pesticide exposure increases α-syn expression and downregulates melanin synthesis upon exposure to ultraviolet (UV) radiation.[18] Aside from environmental factors, common mutations in the parkin genes *PARK2*, *PARK8*, *PARK18*, and *PARK 20* are significantly associated with both PD and melanoma.[19] The products of these genes are involved in the protein dynamics in neurons through autophagia (*PARK8*),[20] synaptic vesicle recycling (*PARK20*),[21] and protein regulation (*PARK2* and *PARK18*).[22] These findings suggest that alterations in protein

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physiology may underlie both cutaneous and neurodegenerative diseases.

The purpose of this study was to compare α-syn presence in skin biopsies from neural crest-derived tissues, including skin from patients with PD; malignant and benign cutaneous lesions, such as melanoma and nevi; skin tags; and healthy skin.

Materials and Methods

Participants

Punch/shave biopsies from patients with melanoma, patients with PD, nevi, skin tags, and healthy donors were obtained. The diagnosis of PD was according to Queen Square Brain Bank criteria. The age-matched control group was comprised of neurologically healthy individuals who agreed to participate in the study. All participants signed the informed consent. The Ethics and Research Committee of the Central Hospital of San Luis Potosi, Mexico, approved the study and the consent forms. Some biopsies from melanoma, nevi, and skin tags were obtained from patients who were having routine excision from a single Dermatology Clinic in Tampa, Florida (R.A.N.). All patients consented to have their biopsies included in this study. One-half of the obtained slides were used for routine stains, and the remaining slides were used for this study.

Immunohistochemistry

All reagents were analytical grade. The biopsies were fixed in 4% paraformaldehyde before they were embedded in paraffin blocks. Five-micrometer tissue slices were collected on electrocharged slides (Biocare Medical LLC, Concord, CA). Slices were dewaxed followed by xylene and ethanol rinses for rehydration. Epitope recovery was done in DIVA decloacker solution (Biocare Medical LLC) in a pressure cooker for 3 minutes. Then, endogenous peroxidase was depleted by incubating with 3% H_2O_2. Then, tissue samples were incubated for 15 minutes with nonspecific background staining blocker (Background Sniper; Biocare Medical LLC) and endogenous biotin and biotin binding proteins blocker (avidin/biotin blocking kit; Vector Laboratories, Inc., Burlingame, CA) alternating with rinses with Tris-buffered saline and Tween-20.

Polyclonal antibodies and a monoclonal anti-α-synuclein antibody were used (Thermo Scientific, Rockford, IL; Becton Dickinson Biosciences, Franklin Lakes, NJ) as well as a monoclonal anti-tyrosinase antibody (ABCAM, Cambridge, MA). Primary antibodies were incubated for 60 minutes, followed by 30 minutes with a streptavidin-biotin detection system (DAKO, Carpinteria, CA). Peroxidase activity was visualized by incubating the sections with amino-ethylcarbazole for 9 minutes to obtain a red coloration and counterstained with Harris hematoxylin. Negative controls consisted of tissue sections treated without the primary antibody. Photomicrographs were taken on a light microscope equipped with a digital camera (Olympus; AmScope, Irvine, CA). Three fields per section were captured at the same magnification (×40) and digitally analyzed using Image Pro Plus 7 software (Media Cybernetics, Rockville, MD). Nonparametric statistical tests (Kruskal-Wallis tests followed by post hoc analyses) were applied to compare the percentage of immunopositive area among the 5 analyzed groups, considering $P < 0.01$ as significant for comparisons between groups.

For immunofluorescence, the primary antibodies were incubated overnight at 4°C, and the secondary antibodies used were a goat anti-mouse immunoglobulin G (IgG) antibody marked with Alexa Fluor 488 and a goat anti-rabbit IgG antibody marked with cyanine 5 Cy5 (Molecular Probes, Eugene, OR). Nuclei were visualized with an orange nucleic acid stain (Sytox; Molecular Probes), and the samples were analyzed with a confocal microscope (LEICA TCS SP2; Leica Microsystems GmbH, Heidelberg, Germany).

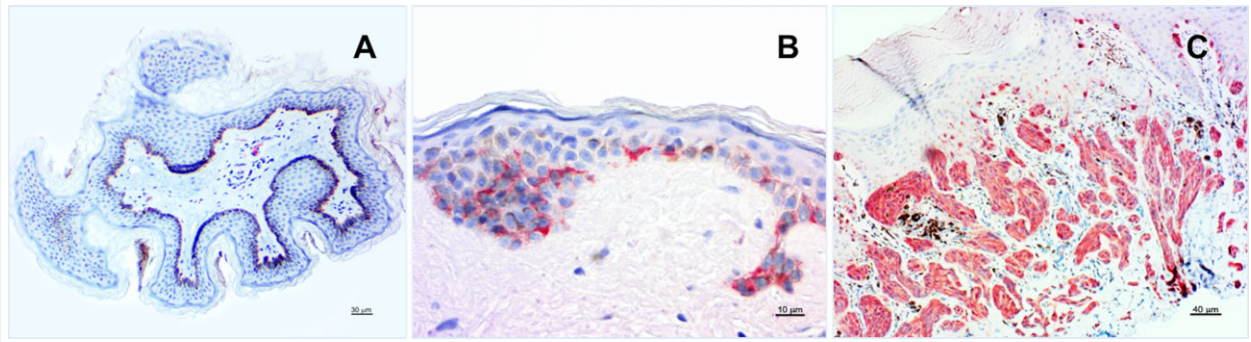

Figure 2 Different patterns of melanin and alpha-synuclein (α-syn) expression in the epidermis. **A:** A skin tag has abundant melanin in the basal layer of the epidermis but scarce α-syn immunopositivity (scale bar = 30 µm). **B:** A sample from a patient with Parkinson's disease has abundant melanin and α-syn in basal and spinous strata of the epidermis. **C:** In melanoma, epithelioid melanocytes are observed at the junction and in the papillary dermis with intense α-syn immunopositivity and some pigmented cells (scale bar = 10 µm).

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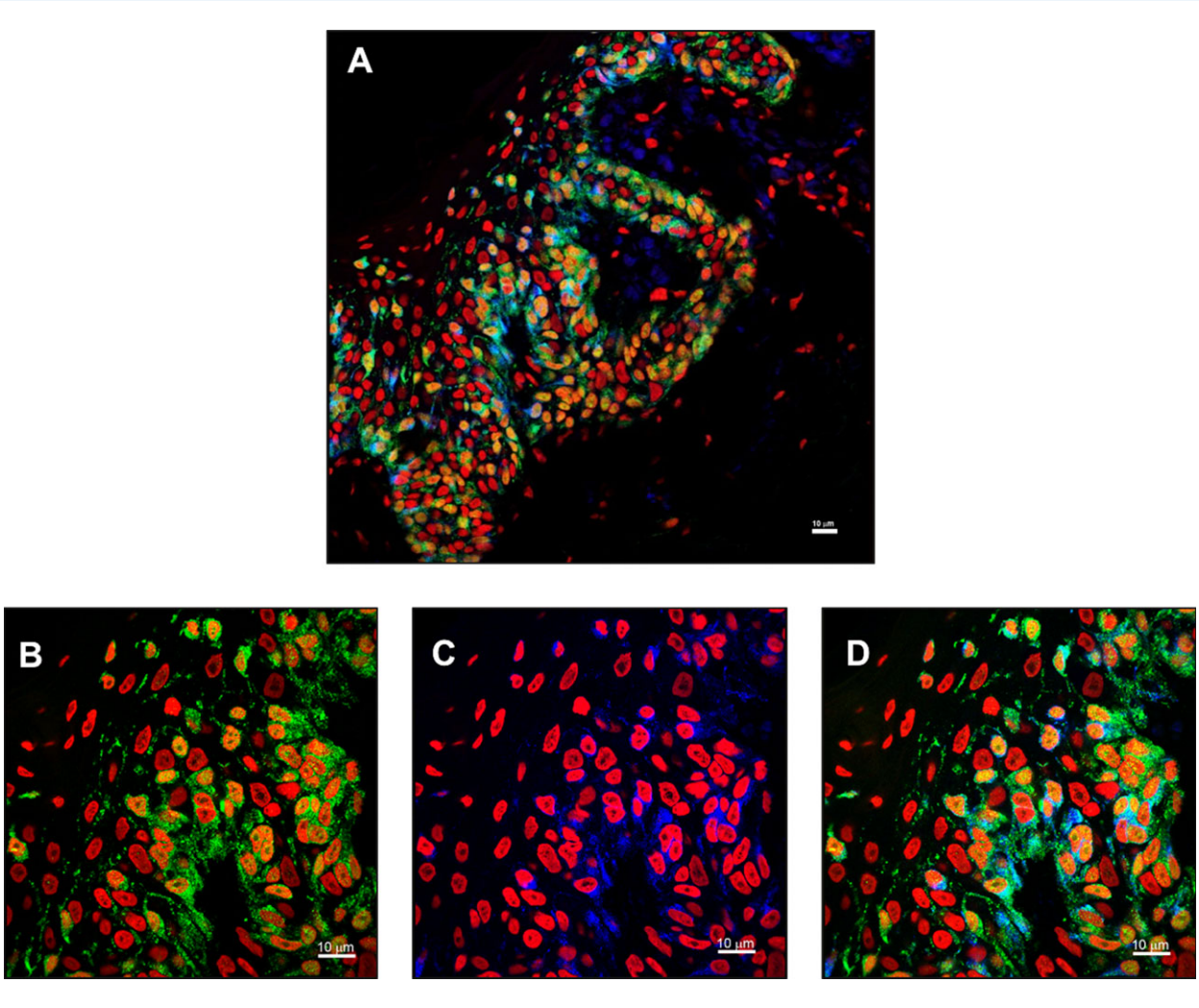

Figure 3 Immunofluorescence patterns of alpha-synuclein (α-syn) and tyrosinase expression in melanoma. **A:** Confocal microscopy reveals α-syn (Alexa Fluor 488; green) and tyrosinase (cyanine 5; blue) antibodies. Cell nuclei were stained with SYTOX (Thermo Fisher Scientific; scale bar = 10 μm). **B:** Panoramic image of a melanoma section. **C:** Immunopositivity for α-syn is localized in cytoplasm, nuclei, and intercellular spaces along the epidermis. **D:** Tyrosinase immunopositivity is restricted to the cytoplasm, mostly in epithelioid melanocytes of the basal layer; this merged image reveals only limited colocalization of α-syn and tyrosinase immunopositivity in basal cells and some keratinocytes.

Results

The main characteristics of the participants are displayed in Table 1. Skin biopsies were obtained from Caucasian, Hispanic, and African American patients. All samples of diagnosed melanoma came from Florida. Racial differences in melanoma are expected in prevalence and incidence due to the origin of samples, but not in phenotype.[23,24]

Photomicrographs of α-syn immunopositivity are provided in Figure 1. In normal skin, scattered basal cells from the epidermis and the protein surrounding cell nuclei are immunopositive (Fig. 1A). In the epidermis of a patient with PD, more cells are immunopositive, and marked particles spread beyond the cell limits, reaching neighboring cells (Fig. 1B). This is more pronounced in nevus cells, where α-syn is also expressed in cells from the stratum spinosum and in the dermis (Fig. 1C); whereas, in melanoma, α-syn immunopositivity is present along the entire epidermis, including keratinocytes from the stratum corneum (Fig. 1D).

Melanin exhibits limited colocalization with α-syn. Figure 2A shows a skin tag with abundant melanin but scattered α-syn staining restricted to basal cells. A similar pattern of α-syn immunopositivity is observed in healthy skin. In the epidermis of patients with PD, however, α-syn immunopositivity is more abundant (Fig. 2B). In a sample of melanoma, clusters of melanocytes are observed invading the dermis and epidermis. Although these cells exhibit strong α-syn immunopositivity, only some groups of cells express melanin (Fig. 2C).

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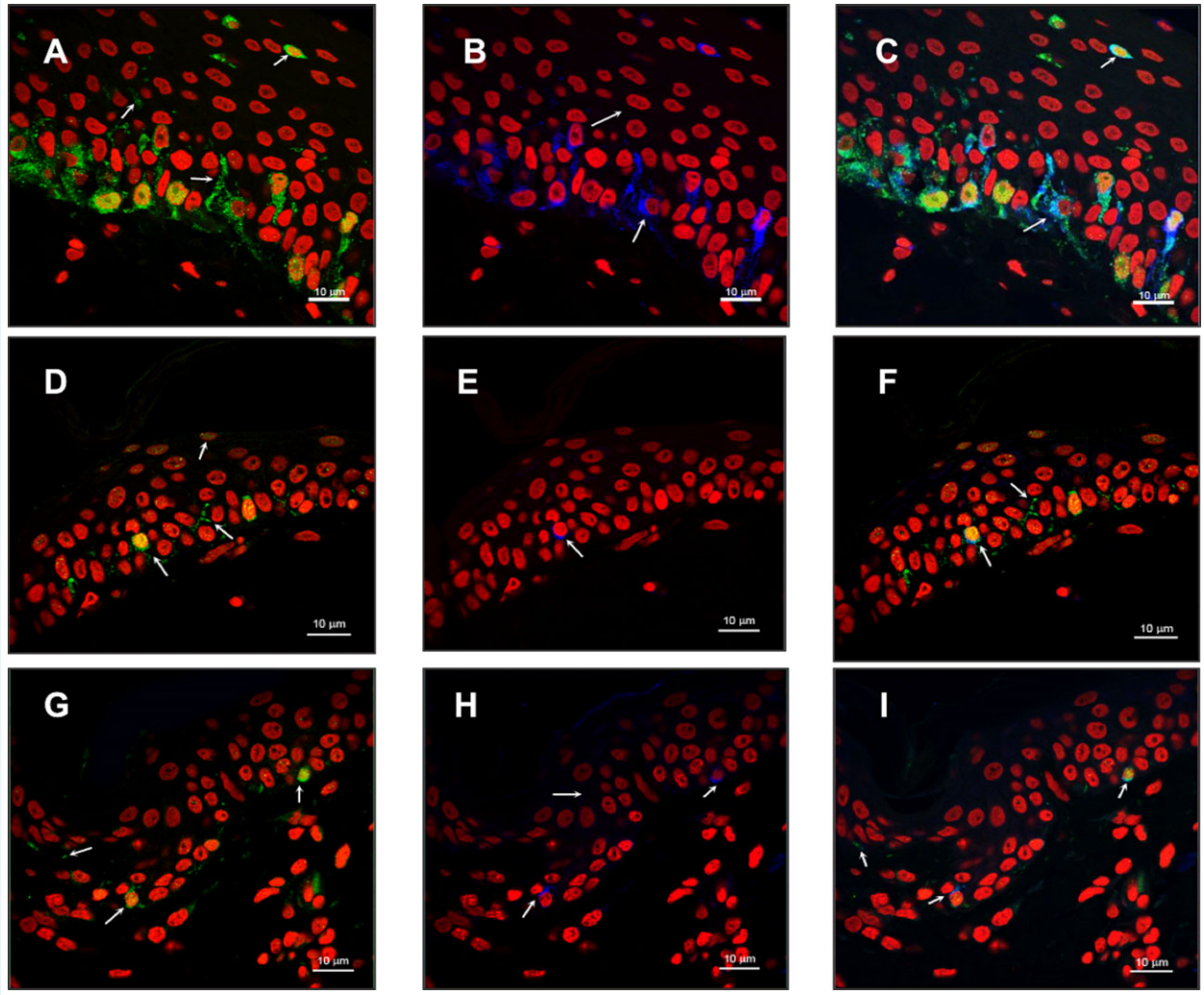

Figure 4 Skin immunofluorescence is observed in nevus (**A**–**C**), Parkinson's disease (**D**–**F**), and healthy controls (**G**–**I**). Arrows in (**A**) and (**D**) indicate the spreading of α-syn along intercellular spaces and nuclei; and arrows in B and E indicate the cytoplasm localization of tyrosinase. **G** and **H:** Scattered immunopositive cells are observed in the basal layer of epidermis from healthy controls. Merged images in (**C**, **F**, and **I**) show the limited colocalization of α-syn and tyrosinase immunopositivity (confocal microscopy; α-syn [Alexa Fluor 488] and tyrosinase [Cy5] antibodies). Cell nuclei were stained with SYTOX. Scale bar = 10 µm.

The possible coexpression of tyrosinase and α-syn was analyzed in detail in a melanoma sample using confocal microscopy. A panoramic view of melanoma is presented in Figure 3A, which reveals abundant expression of α-syn and tyrosinase. α-Syn immunopositivity reveals large particles, suggesting protein aggregates, which are present both in the cytoplasm and nuclei of numerous cells. These granules seem to be located even in intercellular spaces of keratinocytes of the spinous layer (Fig. 3B). Comparatively, tyrosinase-positive cells were less prevalent than α-syn–positive cells, and the staining was limited to the cytoplasm surrounding the nuclei (Fig. 3C). The merged image (Fig. 3D) highlights the different patterns of α-syn and tyrosinase expression. After skin analysis by confocal microscopy of a nevus sample (Fig. 4A–C), a PD sample (Fig. 4D–F), and a healthy control sample (Fig. 4G–H), it can be generalized that α-syn spreads away from cells situated in the stratum basale, reaching keratinocyte nuclei, especially in PD (Fig. 4D), whereas tyrosinase has a more restricted expression to the stratum basale.

The quantification of an α-syn–immunopositive area (ratio of positive area/total area in pixels) in the epidermis is presented in Figure 5. Statistical analysis revealed a significantly higher immunopositivity in PD, nevi, and melanoma samples (3.3 ± 2.3, 7.7 ± 5.4, and 13.6 ± 3.5, respectively) than in healthy controls (0.9 ± 0.7) and skin tags. However, in PD and nevi samples, data indicated a high dispersion, with a median value 3 times and 7 times higher than that in healthy controls, respectively ($P < 0.01$). In all melanoma

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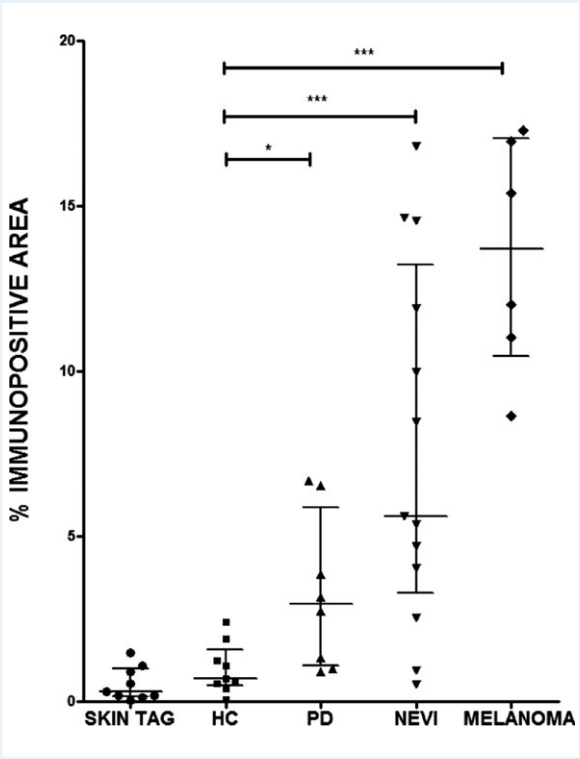

Figure 5 Immunopositivity for alpha-synuclein (α-syn) is expressed as the percentage of immunopositive pixels/total area. Immunoreactivity for α-syn in the Parkison's disease (PD), nevi, and melanoma groups was significantly different from that in healthy controls (HC) (*$P < 0.0125$, ***$P < 0.001$). Bars represent medians and interquartile ranges. Kruskal-Wallis analyses were followed by Mann-Whitney *U* tests.

samples, α-syn immunopositivity values were higher than in any individual skin tag, healthy control, or PD sample ($P < 0.001$).

Discussion

Many epidemiological studies support the association between melanoma and PD as well as the existence of common environmental and genetic risk factors for both diseases. Our proposal was that both PD and melanoma could be manifestations of a proteinopathy, because the affected cells share a common embryologic/neural origin, among other features. The melanocytes and the dopaminergic cells of the substantia nigra produce pigments with a protective purpose. They both produce α-syn, and both have the machinery to export different kinds of molecules. The present work confirmed that there is a higher presence of α-syn in nevi, melanoma, and PD than in healthy skin and other skin diseases, such as skin tags. Also, patients with these 3 conditions seemed to display the spread of α-syn to keratinocytes. In contrast, skin tags and healthy tissues do not exhibit this pattern of α-syn overexpression and localization.

The apparent transference of α-syn to keratinocytes was first observed in the skin of patients with PD. Although healthy individuals express α-syn only in some cells of the stratum basale of the epidermis, α-syn was also present in keratinocytes in biopsies from patients with PD.[13,25] In this work, we propose that α-syn may originate in proliferating melanocytes in the stratum basale (which is then transferred to the adjacent keratinocytes), but that α-syn production is very limited in healthy melanocytes. In samples from melanoma, however, the regulation points of growth and reproduction of melanocytes are lost, and a higher interaction with inflammatory factors takes place.[26] We note that α-syn is present not only in melanocytes but also in basal cells that are negative for tyrosinase. Also, α-syn in keratinocytes seems to be located juxtanuclear and inside the cell nuclei, in contrast to tyrosinase and melanin, which are located exclusively in the cytoplasm (Figs. 3 and 4).This suggests that the physiology of both markers within the cell could not be mutually inclusive.

The presence and transference of α-syn in PD, nevi, and melanoma can be attributed to the ability of neurons and neuroectodermal cells to release toxic proteic aggregates through specialized vesicles called exosomes.[27,28] Also, secreted melanin produced by melanocytes is taken up by surrounding keratinocytes.[5] If melanoma and PD are different final expressions of an initial proteinopathy, then it could be because cells without the ability to reproduce, like those of the CNS, may gradually deteriorate and die[29]; whereas, in the epidermis, the accumulation of α-syn may not lead to cell death but promotes deleterious effects on the cell in response to UV radiation. One example is the reduction of light-induced melanin synthesis through tyrosinase inhibition.[30] There are in vitro data suggesting that the reduction of both melanin content and tyrosinase expression by α-syn overexpression may enhance the susceptibility of skin cells to develop melanoma upon sun exposure,[30] perhaps due to impaired autophagy of the misfolded α-syn and a defective response in UV-damaged cells.

Our study demonstrates that the increase of α-syn immunopositivity in PD, nevi, and melanoma is accompanied neither by a similar tyrosinase increase nor by higher melanin presence. These findings in nevi and melanoma corroborate the previous report by Matsuo and Kamitani.[11] The finding that sunlight and pesticide exposure act synergistically to increase the risk for melanoma and the expression of α-syn in the skin suggests that α-syn may be the link between both diseases. Moreover, there is a possibility that PD could be a condition triggered outside the CNS, as demonstrated by evidence that exposing the enteric system of mice to pesticides[31,32] or curli-producing bacteria (microbiota disruption)[33] results in the aggregation and the neuron-to-neuron spread of α-syn toward the brain.

Another clinical implication of the participation of a protein in neurodegeneration and cutaneous pathology would be the possibility of similar therapeutic targets. Alterations of lysosomal and autophagic functions disrupt α-syn homeostasis and favor the exocytosis of misfolded proteins,[34] and this is followed by an inflammatory response by the microglia.[35] Despite the molecular differences between melanin and neuromelanin, both compounds have a role in cellular

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protection: melanin protects from UV radiation, and neuromelanin is neuroprotective, since it serves as storage for harmful molecules.[36] In the presence of misfolded α-syn and altered autophagy, neuromelanin becomes overloaded and is released from damaged dopaminergic neurons.[8] Both melanoma and PD could benefit from drugs designed to halt the spread of protein aggregates and their noxious interactions with cell organelles and surface receptors, which promote inflammation.[37] A recent study demonstrated a specific interaction of oligomers of phosphorylated α-syn with the mitochondrial membrane. This impairs mitochondrial protein import, which results in deficient mitochondrial respiration and increased production of reactive oxygen species.[37] These findings support a molecular link between PD and mitochondrial dysfunction through α-syn interference, both in the periphery and in the CNS.[38]

In conclusion, by demonstrating intracellular and intercellular α-syn expression in cells of neuroectodermal origin in skin biopsies of patients with PD and melanoma, this study opens experimental and therapeutic avenues of research, providing a molecular link between cutaneous pathology and neurodegeneration through environmental factors like UV radiation and exposure to neurotoxins.

Author Roles

1. Research Project: A. Conception, B. Organization, C. Execution; 2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3. Manuscript Preparation: A. Writing the First Draft, B. Review and Critique.

M.E.J.C.: 1A, IB, IC, 2A, 2B, 3A,3B
I.R.L.: 1C, 3B
E.C.A.: 1C
J.C.R.: 1C
J.P.C.C.: 1C, 3B
M.M.: 1C, 3B
S.S.: 1C, 3B
W.E.: 1C
R.N.: 1C
T.L.: 3B

Disclosures

Ethical Compliance Statement: We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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