



# Exploring the relationship between DUSP3 expression and senescence-associated proliferative status

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## Διερεύνηση της σχέσης μεταξύ της έκφρασης της DUSP3 και της σχετιζόμενης με κυτταρική γήρανση πολλαπλασιαστικής ικανότητας

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### ABSTRACT

**Background and Objectives:** Dual specificity phosphatase 3 (DUSP3) regulates key components of the MAPK/ERK signaling pathway and has been implicated in melanocytic oncogenesis. Given the central role of oncogene-induced senescence in restraining melanocytic tumor progression, the relationship between DUSP3 expression and proliferative fitness remains unclear. This study aimed to investigate whether DUSP3 expression correlates with the proliferative status of melanocytic neoplasms.

**Materials and Methods:** A retrospective immunohistochemical analysis was performed on 172 melanocytic lesions, including common nevi, dysplastic nevi, and paired nevus and melanoma components of nevus-associated melanomas.  $\beta$ -galactosidase expression was used to stratify lesions according to proliferative fitness, and a biologically defined  $\beta$ -galactosidase-negative/low subgroup was analyzed separately.

**Results:** DUSP3 expression was significantly higher in benign melanocytic lesions compared to melanomas, in both the entire cohort and the proliferative fitness subgroup. However, restriction of the analysis to lesions with retained proliferative capacity did not reveal significant differences in DUSP3 expression between them, either overall or within individual diagnostic categories. The relative expression gradient across lesion types remained consistent regardless of senescence status.

**Conclusions:** DUSP3 expression seems to not correlate with proliferative fitness or senescence status. These findings suggest that DUSP3 is not a central regulator of oncogene-induced senescence in melanocytic oncogenesis, and that its role is likely modulatory and context-dependent within broader signaling networks.

**KEYWORDS:** Melanoma, nevi, skin neoplasms, melanocytic, oncogenesis, DUSP3, senescence

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Οι συγγραφείς δηλώνουν ότι δεν υπάρχει σύγκρουση συμφερόντων για το παρόν άρθρο.

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## ΠΕΡΙΛΗΨΗ

**Εισαγωγή και Στόχοι:** Η φωσφατάση διπλής ειδικότητας 3 (DUSP3) ρυθμίζει κομβικά στοιχεία του σηματοδοτικού μονοπατιού MAPK/ERK, ενώ έχει συσχετιστεί με τη μελανοκυτταρική ογκογένεση. Δεδομένου δε του κρίσιμου ρόλου της ογκογονιδιακά επαγόμενης γήρανσης στον περιορισμό της εξέλιξης των μελανοκυτταρικών νεοπλασιών, η σχέση της έκφρασης της DUSP3 με τη διατηρημένη ικανότητα πολλαπλασιασμού παραμένει ασαφής. Σκοπός της μελέτης ήταν η διερεύνηση της συσχέτισης αυτής.

**Υλικά και Μέθοδοι:** Πραγματοποιήθηκε αναδρομική ανοσοϊστοχημική ανάλυση σε 172 μελανοκυτταρικές βλάβες, συμπεριλαμβανομένων κοινών σπύλων, δυσπλαστικών σπύλων και των τμημάτων του σπίλου και του μελανώματος από μελανώματα σχετιζόμενα με σπίλο. Η έκφραση της β-γαλακτοσιδάσης χρησιμοποιήθηκε για τη διαστρωμάτωση των βλαβών ως προς την πολλαπλασιαστική τους ικανότητα, ενώ αναλύθηκε ξεχωριστά υποομάδα με χαμηλή ή αρνητική έκφραση β-γαλακτοσιδάσης.

**Αποτελέσματα:** Η έκφραση της DUSP3 ήταν σημαντικά υψηλότερη στις καλοήθεις μελανοκυτταρικές βλάβες σε σύγκριση με τα μελανώματα, τόσο στο σύνολο του δείγματος όσο και στην υποομάδα με διατηρούμενη πολλαπλασιαστική ικανότητα. Ωστόσο, δεν αναδείχθηκαν στατιστικά σημαντικές διαφορές στην έκφραση της DUSP3 μεταξύ των δύο ομάδων, ούτε συνολικά ούτε εντός των επιμέρους διαγνωστικών κατηγοριών. Η διαβάθμιση της έκφρασης μεταξύ των διαγνωστικών κατηγοριών παρέμεινε σταθερή και στις δύο ομάδες.

**Συμπεράσματα:** Η έκφραση της DUSP3 φαίνεται πως δε συσχετίζεται με την πολλαπλασιαστική ικανότητα ή την κυτταρική γήρανση. Τα ευρήματα υποδηλώνουν ότι η DUSP3 δεν αποτελεί κεντρικό ρυθμιστή της ογκογονιδιακά επαγόμενης γήρανσης στη μελανοκυτταρική ογκογένεση, αλλά πιθανώς δρα με τροποποιητικό και εξαρτώμενο από το βιολογικό πλαίσιο τρόπο.

**ΛΕΞΕΙΣ-ΚΛΕΙΔΙΑ:** Μελάνωμα, σπίλοι, νεοπλάσματα δέρματος, μελανοκυτταρικός, ογκογένεση, γήρανση

## INTRODUCTION

Reversible phosphorylation, controlled through the coordinated actions of kinases and phosphatases, regulates numerous cellular processes and has become a central focus in cancer research. Disruptions in the balance of expression and/or activity between these opposing enzyme groups have been linked to the initiation and progression of many cancers, leading to abnormal phosphorylation of substrates that govern essential cellular functions, including signal transduction, growth, proliferation, differentiation, survival, DNA repair, and stress responses<sup>1,2</sup>. Several of these substrates are known targets of Dual Specificity Phosphatase 3 (DUSP3), underscoring its broad regulatory role across key oncogenic signaling pathways<sup>3-6</sup>.

DUSP3, also referred to as Vaccinia H1-related phosphatase, belongs to the atypical subgroup of the Dual Specificity Phosphatase family and acts on substrates within mitogen- and stress-activated signaling pathways, including ERK, JNK, and p38, thereby regulating cellular responses to external stimuli<sup>7</sup>. DUSP3-mediated dephosphorylation generally suppresses pathway activation and downstream signa-

ling<sup>8,9</sup>. Beyond these functions, DUSP3 has also been implicated in controlling immune and inflammatory responses, as well as maintaining genomic repair and stability<sup>8,9</sup>. However, its limited substrate specificity, the diversity of its targets, and the often conflicting, context-dependent outcomes of its activity make it difficult to categorize DUSP3 strictly as either tumor-promoting or tumor-suppressive<sup>10</sup>. Although proper DUSP3 function appears to support cellular homeostasis and is frequently altered in various cancers, its role may shift depending on the underlying oncogenic drivers—such as mechanisms that bypass oncogene-induced senescence (OIS)—and the stage of tumor progression (benign, malignant, or metastatic)<sup>10-12</sup>. Consequently, both tumor-suppressive and tumor-promoting effects of DUSP3 have been observed across multiple cancer types, including cutaneous melanoma<sup>8-10</sup>.

Based on current evidence concerning the intracellular interactions occurring during melanocytic oncogenesis and DUSP3's established substrates critically participating in it, multiple speculations have been raised supporting either its pro- or anti-tumoral profile<sup>10,13</sup>. DUSP3's role in the oncogenesis of benign and malignant melanocytic tumors has been recently highlight-

ed<sup>14</sup>, however its precise role in melanocytic oncogenesis and its relationship with cellular senescence remain unclear. OIS is recognized as an early tumor-suppressive barrier in melanocytic neoplasia, particularly in lesions driven by activating mutations in BRAF, most commonly BRAF V600E<sup>15</sup>. Constitutive BRAF signaling promotes an initial burst of proliferation in melanocytes but subsequently triggers a stable growth arrest characterized by cell-cycle inhibition, chromatin remodeling, and expression of senescence markers such as senescence-associated  $\beta$ -galactosidase<sup>16</sup>. This senescent state is thought to underlie the benign nature of most melanocytic nevi, where cells harbor oncogenic BRAF yet remain growth-restricted. Progression to malignant melanoma occurs when this senescence program is bypassed or disabled through additional genetic or epigenetic alterations or activation of alternative survival pathways, thereby restoring proliferative capacity and enabling tumor advancement<sup>17-19</sup>.

Given that melanocytic neoplasms are strongly influenced by OIS, and that several established DUSP3 substrates participate in senescence-associated signaling pathways during melanocytic oncogenesis, this study aimed to determine whether DUSP3 expression correlates with the proliferative status of neoplastic melanocytes, further elucidating the role of DUSP3 in melanocytic oncogenesis. Specifically, we analyzed variations in DUSP3 post-translational expression across different types of melanocytic neoplasms exhibiting varying degrees of atypia and distinct biological profiles, yet retaining proliferative capacity and lacking senescence features. The objective of this analysis was to explore the potential involvement of DUSP3 in the regulation or bypass of senescence mechanism.

## MATERIALS AND METHODS

### Data collection

This retrospective study received approval from the Bioethics Committee of the University of Athens. Cases of excised melanocytic neoplasms were identified through the biopsy archives of the 1st Department of Pathology, National and Kapodistrian University of Athens, and the Department of Pathology at Andreas Syggros Hospital. Inclusion criteria comprised: (i) histopathological diagnoses of common acquired melanocytic nevus (CN), dysplastic acquired melanocytic nevus (DN), irrespective of the degree of atypia, or cutaneous invasive nevus-associated melanoma (NAM); (ii) confirmation of these

diagnoses and assessment of tissue suitability through independent review of hematoxylin and eosin-stained slides by two pathologists, including clear demarcation between nevus and melanoma areas in NAM specimens; (iii) availability of representative paraffin-embedded tissue blocks for further analysis; and (iv) sufficient accompanying demographic information. Lesions exhibiting spitzoid or congenital characteristics were excluded. Based on these criteria, four tissue subgroups were established: CN, DN, and the nevus and melanoma components of NAM (designated N-NAM and M-NAM, respectively). Although the latter two groups originated from the same NAM specimens, they were analyzed independently for study purposes. Patient sex, age at diagnosis, and histological subtype of nevi were also recorded.

### Processing

Immunohistochemical staining for the markers of interest was carried out on 4- $\mu$ m-thick formalin-fixed, paraffin-embedded tissue sections. Following overnight incubation at 37°C, deparaffinization, rehydration, and antigen retrieval were performed simultaneously using an automated PT Link system (Dako), with slides treated for 20 minutes at 96°C in EnVision FLEX Target Retrieval Solution High pH (50 $\times$ ) (Dako, Denmark). Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide prepared in Tris-buffered saline (TBS) for 15 minutes. After rinsing with TBS, sections were incubated for 1 hour with a rabbit polyclonal anti-DUSP3 antibody (LifeSpan Biosciences, Seattle, WA) at a 1:500 dilution. Parallel sections from the same lesions were also incubated for 1 hour with a mouse monoclonal anti- $\beta$ -galactosidase antibody (clone OT11E10; LifeSpan Biosciences) at a 1:200 dilution. Antibody detection was performed using a two-step polymer-based visualization system (goat anti-mouse/rabbit IgG-HRP; ImmunoLogic, WellMed BV, The Netherlands). Slides were first treated with a post-blocking solution for 30 minutes at room temperature, followed by incubation with the HRP-conjugated polymer for 30 minutes. Signal development was achieved using the EnVision FLEX HRP Magenta Substrate Chromogen System (Dako, Denmark). Finally, sections were counterstained with hematoxylin and mounted for evaluation.

### Evaluation of immunohistochemical staining for DUSP3

Two independent observers (EC and NK) evaluated the

immunohistochemical staining using light microscopy, assessing and documenting two distinct parameters for each case: (i) staining intensity within the examined lesion, graded as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong); and (ii) staining extent, defined as the proportion of positively stained lesional cells, expressed as a percentage ranging from 0% to 100%. Any discrepancies between the reviewers were resolved through joint re-evaluation and consensus. Immunoreactivity was quantified using a modified Immunoreactive Score (IRS)<sup>20,21</sup>, previously applied in the assessment of DUSP2 expression in ovarian carcinoma<sup>22</sup>. For each specimen, a composite score was calculated by multiplying the intensity grade by the extent score. For this purpose, percentage values for staining extent were categorized as follows: 0% = 0, 1–25% = 1, 26–50% = 2, 51–75% = 3, and 76–100% = 4. Accordingly, each case was assigned to a positivity category based on the final calculated score as follows: scores of 0–1 were considered negative, 2–3 indicated mild positivity, 4–8 corresponded to moderate positivity, and 9–12 were classified as strong positivity<sup>20</sup>.

### Evaluation of immunohistochemical staining for B-galactosidase

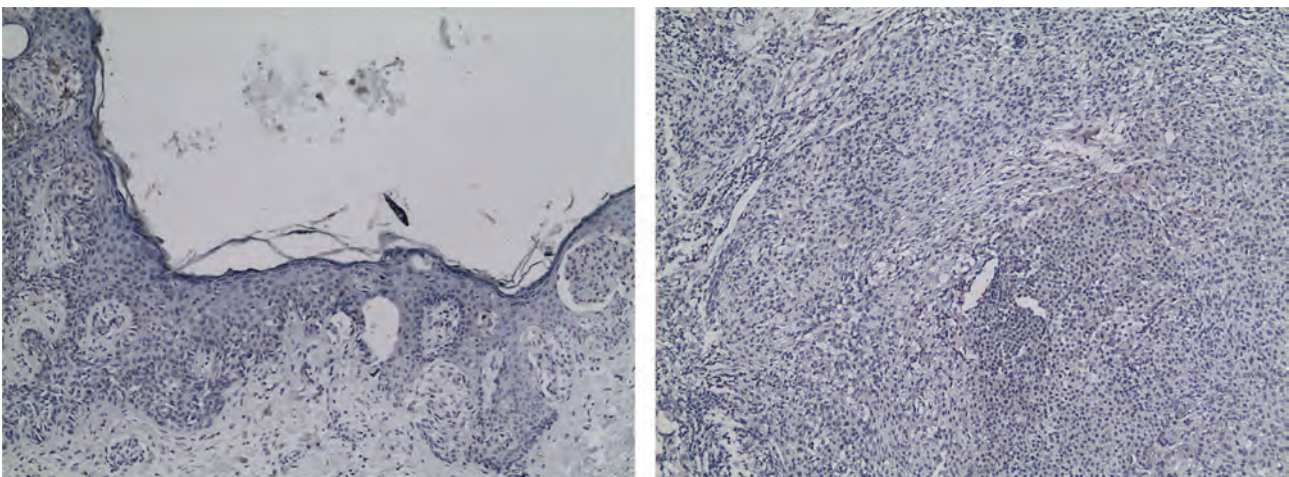
Two independent observers (EC and NK) evaluated the immunohistochemical staining using light microscopy, assessing and documenting staining intensity within the examined lesion, graded as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong) for each case. Any discrepancies between the reviewers were resolved through joint re-evaluation and consensus.

### B-Galactosidase-Based stratification

Accordingly, from the total cohort, we isolated a biologically defined subgroup of lesions exhibiting low  $\beta$ -gal intensity (grade 0–1), hereafter referred to as the proliferative-fitness subgroup. This approach allowed focused evaluation of DUSP3 expression independently of advanced senescence-related metabolic alterations. Senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) activity constitutes a well-established histochemical surrogate of oncogene-induced and replicative senescence in melanocytic neoplasms. Increased  $\beta$ -gal staining intensity reflects lysosomal expansion and metabolic remodeling characteristic of senescent, replication-incompetent melanocytes, whereas absent or weak staining is indicative of preserved proliferative fitness<sup>23</sup>. Given the pivotal role of senescence as a pivotal tumor-suppressive barrier in melanocytic oncogenesis, lesions exhibiting negative (grade 0) or weak (grade 1)  $\beta$ -gal staining were considered representative of melanocytic populations retaining proliferative competence. Representative immunohistochemical images are provided in Figure 1.

### Statistical analysis

Normality of the data was evaluated using the Shapiro–Wilk test. Continuous variables are reported as mean  $\pm$  standard deviation (SD) for normally distributed data or as median with interquartile range (IQR) for non-normally distributed data, while categorical variables are expressed as absolute frequencies and percentages. For normally distributed variables, comparisons between two groups were performed using the



**FIGURE 1** | Images of the nevus component (N-NAM, left) and melanoma component (M-NAM, right) of the same NAM, weakly stained for  $\beta$ -gal. Both neoplasms were included in the proliferative fitness subgroup. 100x magnification.

independent samples t-test, with or without Welch’s correction as appropriate, whereas comparisons among three or more groups were conducted using one-way ANOVA followed by Dunnett’s post hoc test. For non-normally distributed variables, the Mann–Whitney U test was used for two-group comparisons and the Kruskal–Wallis test followed by Dunn’s multiple comparisons test for analyses involving three or more groups. All statistical tests were two-tailed, and analyses were performed using IBM SPSS Statistics version 28.0.

## RESULTS

We conducted a retrospective analysis of 172 melanocytic neoplasm cases obtained from 130 patients, including 42 CN, 46 DN, and 84 paired samples representing the nevus and melanoma components of 42

NAMs. Of the 130 patients, 56 were male and 72 were female, with sex data unavailable for two individuals. The median age was 44 years (range 15–86; IQR 31 years). Among the 130 nevi evaluated, 88 (67.7%) were junctional, 39 (30.0%) were compound, and 3 (2.3%) were dermal. Detailed sample characteristics are summarized in Table 1.

Focusing on the proliferative fitness subgroup, 43 melanocytic lesions with retained proliferative competency from 39 patients were isolated from the entire sample, comprising 13 CN, 7 DN, 6 N-NAMs, and 17 M-NAMs. Among the 39 patients, 18 were male and 20 were female, with sex information unavailable for one individual. The overall median age at diagnosis was 55 years (range 18–85; IQR 37.5 years). When stratified by diagnosis, the median age was 36 years for CN (range 21–45; IQR 17.5), 65 years for DN (range 38–76; IQR 38), 62 years for N-NAMs (range 18–85; IQR 20), and 72 years for M-NAMs (range 18–85; IQR 25). Of the 26

**TABLE 1** Characteristics of the cases/individuals included in the entire study sample.

Characteristics	
Diagnosis (n=172 lesions, 0 missing)	N
CN	42
DN	46
N-NAMs	42
M-NAMs	42
Sex (n=128 individuals, 2 missing)	N (%)
Male	56 (44%)
Female	72 (56%)
Age at diagnosis (n=130 individuals, 0 missing)	Median (range, IQR), in years
Total	44 years (range: 15-86, IQR: 31)
CN	38.5 (range: 15-86, IQR: 21)
DN	38 (range: 16-79, IQR: 22)
NAMs	62 (range: 18-85, IQR: 20)
Nevus Subtype (n=130, 0 missing)	N (%)
Junctional	88 (67.7%)
Mixed	39 (30.0%)
Dermal	3 (2.3%)



nevi assessed for histologic subtype, 23 (88.5%) were junctional and 3 (11.5%) were mixed, while no dermal nevi were identified. Detailed characteristics are presented in Table 2.

### Descriptive analysis of DUSP3'S expression for the entire sample

The immunohistochemical expression of DUSP3 was assessed using the IRS-based positivity classification (categorical), as previously described, which categorized staining into four levels: negative, mild, moderate, and strong. The findings are summarized in Table 3. Overall, nevi exhibited higher positivity than M-NAMs, with most CN, DN, and N-NAM cases showing moderate staining intensity (61.9%, 54.3%, and 57.1%, respectively). In contrast, the majority of M-NAMs displayed only mild positivity (52.4%). This pattern was also reflected in the mean IRS scores for each group, where benign melanocytic lesions demon-

strated higher DUSP3 expression than malignant ones [mean (SD) IRS scores: CN 7.5 (3.5); DN 6.2 (3.6); N-NAMs 4.0 (2.9); M-NAMs 1.9 (1.1),  $p < 0.001$ ]. Representative immunohistochemical images are provided in Figure 2. Negative staining was most frequently observed in M-NAMs and least frequently in CN, whereas strong staining was most common in CN and rarest in M-NAMs, with no M-NAMs showing strong positivity. Moreover, most NAMs (57.1%) arose from nevi with moderate positivity, while a notable proportion (21.4%) developed from negative lesions and only a small fraction (4.8%) originated from strongly positive nevi.

### Descriptive analysis of dusp3's expression for the proliferative fitness subgroup

Within the  $\beta$ -gal-negative/low subgroup, DUSP3 expression was evaluated using the same IRS methodology applied to the entire cohort. The findings are sum-

**TABLE 2** Characteristics of the cases/individuals included in the proliferative fitness subgroup.

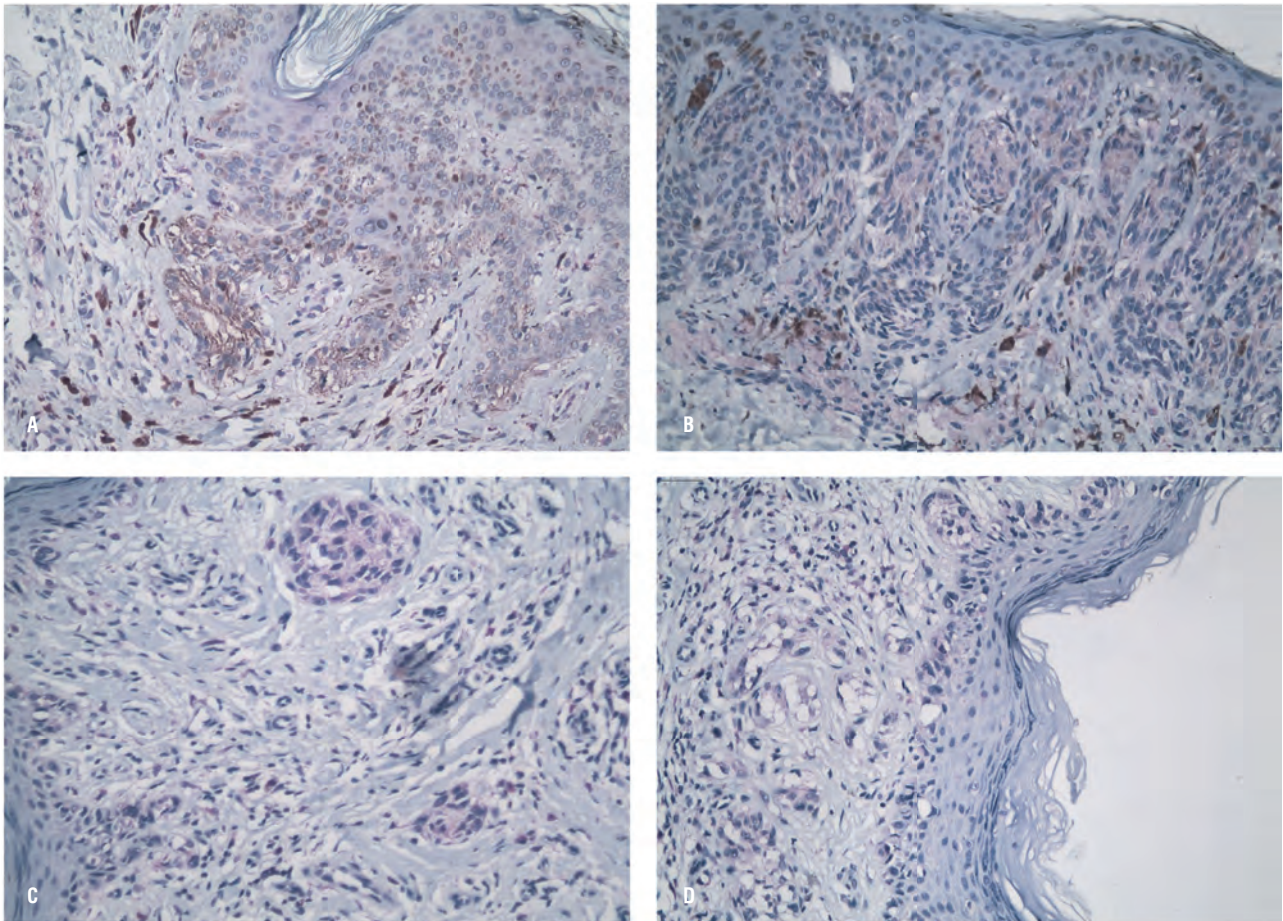
Characteristics	
Diagnosis (n=43 lesions, 0 missing)	N
CN	13
DN	7
N-NAMs	6
M-NAMs	17
Sex (n=39 individuals, 1 missing)	N (%)
Male	18 (46%)
Female	20 (54%)
Age at diagnosis (n=39 individuals, 0 missing)	Median (range, IQR), in years
Total	55 (range: 18–85, IQR: 37.5)
CN	36 (range: 21–45, IQR: 17.5)
DN	65 (range: 38–76, IQR: 38)
N-NAMs	62 (range: 18–85, IQR: 20)
M-NAMs	72 (range: 18–85, IQR: 25)
Nevus Subtype (n=26, 0 missing)	N (%)
Junctional	23 (88.5%)
Mixed	3 (11.5%)
Dermal	0 (0%)

**TABLE 3** A descriptive overview of the distribution of cases of the entire sample for each diagnosis across the DUSP3 IRS score categories is presented. Values are given as absolute counts and percentages [N (%)]. The IRS score categories for positivity are defined as follows: 0–1 = negative, 2–3 = mild positivity, 4–8 = moderate positivity, and 9–12 = strong positivity.

		Categorical DUSP3's IRS score [N (%)]					Numerical DUSP3's IRS score	
		negative	mild	moderate	strong	Total	mean	SD
Diagnosis	CN	3 (7.1%)	2 (4.8%)	26 (61.9%)	11 (26.2%)	42 (100.0%)	7.5	3.5
	DN	4 (8.7%)	8 (17.4%)	25 (54.3%)	9 (19.6%)	46 (100.0%)	6.2	3.6
	N-NAMs	9 (21.4%)	7 (16.7%)	24 (57.1%)	2 (4.8%)	42 (100.0%)	4.0	2.9
	M-NAMs	16 (38.1%)	22 (52.4%)	4 (9.5%)	0 (0.0%)	42 (100.0%)	1.9	1.1
	Total	32 (18.6%)	39 (22.7%)	79 (45.9%)	22 (12.8%)	172 (100.0%)	5.0	3.6

marized in Table 4. Overall, nevi (CN, DN, and N-NAMs) exhibited higher DUSP3 expression than M-NAMs. Specifically, most CN and DN cases showed moderate

staining intensity (CN 46.2%, DN 28.6%), with a notable proportion of CN also showing strong positivity (30.8%). N-NAMs also largely demonstrated moderate



**FIGURE 2 |** Images of lesions from the entire sample with IRS scores representative of their respective group means, immunohistochemically stained for DUSP3. 200x magnification. A: CN with DUSP3 IRS=8; B: DN with DUSP3 IRS=6; N-NAM with DUSP3 IRS=4; M-NAM with DUSP3 IRS=2.

**TABLE 4** A descriptive overview of the distribution of cases of the proliferative fitness subgroup for each diagnosis across the DUSP3 IRS score categories is presented. Values are given as absolute counts and percentages [N (%)]. The IRS score categories for positivity are defined as follows: 0–1 = negative, 2–3 = mild positivity, 4–8 = moderate positivity, and 9–12 = strong positivity.

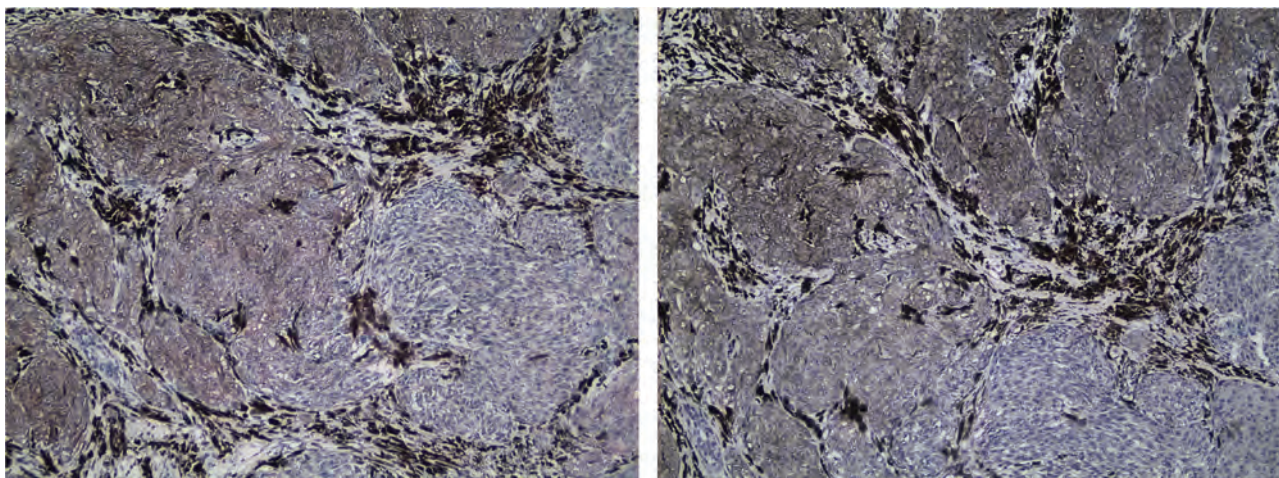
		Categorical DUSP3's IRS score [N (%)]					Numerical DUSP3's IRS score	
		negative	mild	moderate	strong	Total	mean	SD
Diagnosis	CN	1 (7.7%)	2 (15.4%)	6 (46.2%)	4 (30.8%)	13 (100.0%)	6.9	4.2
	DN	1 (14.3%)	2 (28.6%)	2 (28.6%)	2 (28.6%)	7 (100.0%)	6.6	4.6
	N-NAMs	1 (16.7%)	1 (16.7%)	4 (66.7%)	0 (0%)	6 (100.0%)	3.2	1.3
	M-NAMs	9 (53.0%)	6 (35.3%)	2 (11.8%)	0 (0%)	17 (100.0%)	1.8	1.3
	Total	12 (27.9%)	11 (25.6%)	14 (32.6%)	6 (14.0%)	43 (100.0%)	4.3	3.8

positivity (66.7%). On the contrary, M-NAMs predominantly displayed negative staining (53.0%), as shown in Figure 3, and were frequently mildly positive (35.3%). This trend was mirrored in the mean IRS scores, where benign lesions had higher expression than malignant ones [mean (SD) IRS scores: CN 6.9 (4.2); DN 6.6 (4.6); N-NAMs 3.2 (1.3); M-NAMs 1.8 (1.3),  $p < 0.001$ ]. Negative staining was most prevalent in M-NAMs and least common in CN, whereas strong staining was observed mainly in CN and was absent in M-NAMs.

### Association of DUSP3's expression with proliferative fitness

Comparison of DUSP3 expression between the entire sample and the subgroup of tumors with retained pro-

liferative fitness was performed. For each diagnostic category, mean numerical DUSP3 IRS scores of the proliferative fitness subgroup were compared with those of the corresponding full-sample group, as shown in Table 5. No statistically significant differences were observed for any diagnostic category (all  $p > 0.05$ ). Considering the proliferative fitness subgroup as a whole, the mean IRS score was  $4.33 \pm 3.80$ , compared with  $5.0 \pm 3.6$  in the entire cohort, a difference that also did not reach statistical significance ( $p > 0.05$ ). These findings indicate that overall DUSP3 expression is not substantially altered when analysis is restricted to melanocytic lesions retaining proliferative fitness. Importantly, mean IRS values within each subgroup were directionally consistent with those of the entire cohort and showed no systematic upward or downward shifts.



**FIGURE 3** | Characteristic image of an M-NAM from the proliferative fitness subgroup, negative for both  $\beta$ -gal (left) and DUSP3 (IRS=1) (right). 100x magnification.

**TABLE 5** Mean values of numerical DUSP3's IRS score for the two study groups.

		Numerical DUSP3's IRS score for the entire sample		Numerical DUSP3's IRS score for the proliferative fitness subgroup		Statistical significance
		mean	SD	mean	SD	
Diagnosis	CN	7.5	3.5	6.9	4.2	p > 0.05
	DN	6.2	3.6	6.6	4.6	p > 0.05
	N-NAMs	4.0	2.9	3.2	1.3	p > 0.05
	M-NAMs	1.9	1.1	1.8	1.3	p > 0.05
	Total	5.0	3.6	4.3	3.8	p > 0.05

## DISCUSSION

OIS, particularly BRAF-induced OIS, represents a critical biological checkpoint that separates benign melanocytic proliferations from malignant transformation<sup>15</sup>. Constitutive activation of the MAPK/ERK pathway, most commonly driven by BRAF mutations, characterizes both benign and malignant melanocytic neoplasms and necessitates tight regulatory control to maintain cellular homeostasis. DUSP3 is thought to be among the negative regulators of this pathway, as it dephosphorylates and inhibits two key substrates within the cascade: the upstream receptor tyrosine kinases ERBB1/2 (EGFR1/2) and the terminal cytosolic effectors ERK1/2<sup>3</sup>. Through this activity, DUSP3 is positioned to modulate both pathway initiation and downstream signaling progression, implicating it as a potential mediator of proliferation-senescence balance during melanocytic oncogenesis<sup>10</sup>. The observation that DUSP3 presence -and possibly functional competence- is more frequently associated with senescent or less hyperproliferative lesions, such as common and dysplastic nevi, than malignant melanocytic tissues suggests a putative protective role against malignant transformation<sup>14</sup>. Such an effect could reflect a compensatory response to oncogenic MAPK/ERK overactivation, contributing to appropriate cell-cycle regulation and maintenance of an OIS phenotype. Conversely, reduced DUSP3 expression might attenuate this oncosuppressive influence, facilitating escape from growth control and predisposing neoplastic populations to hyperproliferation, a potential early step toward melanoma development<sup>10</sup>.

However, the biological role of DUSP3 appears complex and potentially context-dependent. Experi-

mental evidence indicates that DUSP3 is required for normal cell-cycle progression and mitosis, with its loss promoting senescence<sup>11</sup>. Consistently, stronger immunohistochemical expression has been documented in dysplastic nevi (lesions characterized by higher proliferative activity) compared to common nevi, as well as within their less mature, proliferative epidermal compartments of nevi<sup>24</sup>. These findings suggest that, while DUSP3 may physiologically regulate proliferation through modulation of MAPK/ERK signaling, its activity in the setting of sustained pathway hyperactivation could paradoxically interfere with the establishment or maintenance of OIS. Collectively, these observations raise the possibility that DUSP3 may influence melanocytic tumor biology not only by restraining oncogenic signaling but also by modulating the threshold for OIS induction or bypass.

Given these considerations, the present study aimed to further clarify the role of DUSP3 in melanocytic oncogenesis from the perspective of OIS regulation and maintenance, acknowledging its established importance in physiological cell-cycle control and its recently described potential oncosuppressive function<sup>14</sup>. We specifically investigated whether DUSP3 expression correlates with proliferative fitness across benign and malignant melanocytic lesions of various types.

Restriction of the analysis to lesions retaining proliferative competence, defined by absent or weak  $\beta$ -galactosidase activity, did not reveal significant differences in DUSP3 expression compared to lesions with variable proliferative status, either overall or within individual diagnostic categories. This lack of association argues against a direct mechanistic role for DUSP3 in the in-



duction or maintenance of the senescent phenotype or in senescence-associated metabolic remodeling. The absence of divergence in DUSP3 expression among proliferatively competent lesions further indicates that DUSP3 levels do not adapt to the presence or absence of senescence. In other words, replicative status does not seem to influence DUSP3 expression regardless of the melanocytic neoplasm type. Instead, DUSP3 expression appeared more closely related to tumor type-specific biological characteristics rather than replicative status. Supporting this interpretation, the relative gradient of expression across diagnostic groups within the proliferative fitness subgroup mirrored that of the entire cohort, with nevi exhibiting higher levels and melanomas the lowest, independent of senescence markers.

Moreover, the identification of benign lesions (nevi) capable of sustained proliferation despite the putative protective barrier imposed by elevated DUSP3 expression suggests that melanocytic tumors may rely on DUSP3-independent mechanisms to maintain cell-cycle progression. This observation raises the possibility that alternative regulatory pathways compensate for increased DUSP3 activity, enabling a functional bypass of DUSP3-mediated control, or that the effects of DUSP3 vary in the context of benign oncogenesis, potentially facilitating OIS bypass<sup>17,25</sup>. In contrast, malignant lesions (NAMs) that retain proliferative capacity consistently exhibit reduced DUSP3 expression, supporting the notion that DUSP3 indeed exerts a protective role in malignant scenarios<sup>14</sup>.

To our knowledge, this is the first study to examine

DUSP3 expression in relation to proliferative status in melanocytic oncogenesis. However, the analysis was limited to immunohistochemical assessment of DUSP3, without evaluation of its pre-translational regulation, including genetic alterations, epigenetic modifications, or transcriptional control, nor its specific functional activity. Additional limitations should be acknowledged. Our conclusions regarding melanoma primarily apply to the superficial spreading subtype, which is more commonly associated with preexisting nevi<sup>26,27</sup>. Although  $\beta$ -galactosidase is a robust and widely accepted marker of melanocytic senescence, reliance on a single marker represents an inherent limitation. Incorporation of additional markers would allow for more precise stratification of proliferative and senescent phenotypes. Moreover, integration of molecular and functional assays could further clarify the role of DUSP3 within senescence-associated signaling networks. Finally, additional studies are warranted to validate these findings by examining DUSP3 expression in well-defined cohorts of senescent melanocytic lesions.

In conclusion, while DUSP3 clearly participates in MAPK/ERK pathway regulation and physiological cell-cycle progression, our findings do not support a central or deterministic role for DUSP3 in governing OIS in melanocytic neoplasia. Rather, its contribution may be modulatory and context-dependent, embedded within broader, redundant signaling networks that collectively determine whether melanocytic cells undergo senescence or continue proliferating during tumor evolution.

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