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Subcellular Localization of PDCD4 in Melanoma and Its Prognostic Implications in Tumor and Immune Compartments

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ABSTRACT

The function and subcellular localization of programmed cell death 4 (PDCD4) in melanoma remain poorly characterized. Previous work suggests PDCD4 interacts with Pleckstrin Homology Domain Containing A5 (PLEKHA5), influencing survival in patients with melanoma brain metastases. Here, we investigated the distribution of PDCD4 within tumor cells and the surrounding microenvironment and examined its association with clinical outcomes. Using quantitative immunofluorescence on tissue microarrays with comprehensive clinicopathological annotations, combined with single-cell RNA sequencing of a brain metastasis, we profiled PDCD4-positive immune cell populations. Our results reveal stage-specific changes in PDCD4 expression, with higher levels in tumors and stroma linked to improved survival in primary melanomas and intracranial metastases, but not in extracranial metastatic lesions. Beyond its known expression on CD8+ T cells and natural killer cells, PDCD4 was also present on B cells and mast cells. Elevated PDCD4 within the tumor microenvironment correlated with increased immune infiltration. These findings highlight the potential importance of the PDCD4–PLEKHA5 axis in melanoma brain metastasis and support further exploration of this pathway as a therapeutic target.

Keywords: PDCD4, Melanoma, Brain metastasis, PLEKHA5, Tumor-infiltrating immune cells

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Introduction

Melanoma accounts for the majority of deaths caused by skin cancer and exhibits a strong tendency to spread to the brain [1]. Over the last decade, advances in systemic and local therapies—including targeted agents, immune checkpoint inhibitors, and stereotactic radiosurgery—have improved survival for patients with melanoma brain metastases. However, these treatments are only partially effective due to limited drug delivery across the blood–brain barrier and treatment-related toxicities [2–12]. Despite progress, few molecular mechanisms have been specifically linked to melanoma's preference for the brain, highlighting a critical gap in our understanding [13–21]. Identifying the molecules and signaling pathways driving brain metastasis is essential for discovering new therapeutic targets and developing strategies to overcome resistance.

Our prior work identified Pleckstrin Homology Domain Containing A5 (PLEKHA5), a gene involved in normal brain development, as a key regulator of melanoma growth in the brain [22]. We found that PLEKHA5 affects melanoma progression through modulation of programmed cell death 4 (PDCD4), a cell cycle inhibitor, via interactions with the ubiquitin-proteasome system and PI3K/AKT/mTOR signaling pathways [22–28]. Analyses of paired cranial and extracranial melanoma samples revealed that higher PDCD4 expression in brain lesions correlates with improved survival, suggesting that loss of PDCD4 may promote melanoma brain metastasis [22]. PDCD4, originally identified on chromosome 10q24 as a nuclear antigen gene [29], functions as a tumor suppressor and is frequently downregulated or lost in multiple cancers, including melanoma, lung, liver, breast, colorectal, and gastric cancers [23, 29, 30]. Its expression is regulated by multiple pathways, and its biological effects vary by cell type [23, 29]. PDCD4 can inhibit tumor initiation, angiogenesis, invasion, and induce apoptosis

[23, 29]. Reduced PDCD4 expression in tumor cells is also linked to resistance to chemotherapy and radiotherapy, whereas its presence can enhance treatment sensitivity [24, 31–34]. Beyond its role in cancer, PDCD4 regulates inflammation, with lower expression associated with an anti-inflammatory profile [29, 35]. MicroRNA-mediated PDCD4 suppression promotes IL-10 production and reduces pro-inflammatory cytokines such as IL-6 and TNF-α. PDCD4 is also expressed in cytotoxic T cells, where it is regulated by CTLA-4 and influences T-cell differentiation [36].

Loss or decreased expression of PDCD4 is associated with tumor progression and poor prognosis in multiple malignancies, including cancers of the head and neck, brain, breast, lung, digestive, reproductive, and urinary systems [23–28, 37]. In melanoma, however, the prognostic significance and clinicopathological impact of PDCD4 expression remain poorly understood. Limited studies have shown that low PDCD4 mRNA levels in primary melanomas correlate with larger tumor size, higher Clark levels, and lymph node involvement [30]. Our previous work using quantitative immunofluorescence (QIF) demonstrated that low or absent PDCD4 protein in cerebral melanoma metastases is associated with shorter patient survival [22].

PDCD4 localizes to both the nucleus and cytoplasm of normal and malignant cells, shuttling between compartments. Loss of nuclear localization and overall PDCD4 expression has been observed during melanoma progression [38, 39]. In our prior study, PDCD4 staining was predominantly cytoplasmic in brain metastases compared with paired extracranial metastases, but the small cohort (n = 37) limited the ability to assess the prognostic relevance of subcellular patterns [22]. Most previous studies have only examined total PDCD4 protein levels, with very few evaluating nuclear versus cytoplasmic distribution [37, 38]. Accurate assessment of PDCD4 localization at different disease stages is therefore crucial for understanding its clinical significance. Additionally, PDCD4 expression in the tumor microenvironment remains largely unexplored.

In this study, we aimed to comprehensively analyze PDCD4 expression in human melanoma and benign nevi, examining its distribution in tumor cells and tumor-infiltrating immune cells. Using a large, well-annotated cohort of primary and metastatic melanoma specimens, we sought to define the prognostic role of PDCD4 in both tumor and immune compartments.

Materials and Methods

Tissue microarray (TMA) preparation

Paraffin-embedded tissue blocks from melanoma and nevi were collected from the Yale University Pathology Archives with Institutional Review Board approval. Representative tumor regions were identified by a pathologist, and 0.6 mm cores were taken and arranged into tissue microarrays.

The main melanoma TMA comprised 230 primary and 293 metastatic melanomas resected between 1959 and 2000. Patients were 55% male, with an average age of 52.4 years (range 18–91), and mean follow-up of 6.7 years (range 2 months to 40 years). The nevus TMA included 263 benign lesions and 40 melanoma samples overlapping with the melanoma cohort. Identical cell line cores were included on both arrays to normalize staining and scoring.

A separate paired cerebral–extracranial melanoma TMA contained 37 patients who underwent craniotomy between 1997 and 2014. Patient demographics and clinical characteristics were previously described [40]. Mean age at diagnosis was 51 years (range 19–78), with 68% male. Survival was calculated from first distant metastasis to death or last follow-up (mean 1.8 years) and from first melanoma brain metastasis to death or last follow-up (mean 1.2 years). Brain metastasis-free survival was measured from stage IV diagnosis to detection of brain metastases (mean 0.52 years).

Immunofluorescent staining of PDCD4

Five-micrometer TMA sections were mounted on slides using adhesive tape-transfer with UV crosslinking. Sections were deparaffinized, rehydrated, and subjected to antigen retrieval in Tris-EDTA buffer (pH 8.0) using a pressure cooker for 20 minutes. Endogenous peroxidase was quenched with hydrogen peroxide, and non-specific binding was blocked with 0.3% bovine serum albumin.

Slides were incubated with rabbit anti-PDCD4 (Cell Signaling) and either mouse anti-S100/HMB45 (BioGenex) to identify tumor cells, or anti-CD3 (Leica) for T cells. Additional serial sections were stained with antibodies against CD4, CD8, FOXP3, CD20, and CD68 to assess immune subsets. Signal amplification used Envision goat anti-rabbit or anti-mouse reagents (Dako), and visualization was performed with Cyanine-3 or Cyanine-5 tyramides (Perkin Elmer). S100/HMB45 staining was visualized using Alexa 546-conjugated secondary antibody.

Nuclei were counterstained with DAPI, and slides were mounted with ProLong Gold antifade medium (Invitrogen).

Quantification of PDCD4 expression

Fluorescence images were captured and analyzed using previously described methods [40–42]. Tumor and stromal compartments were distinguished using S100/HMB45 and DAPI masks. PDCD4 signal intensity was measured in tumor, stroma, and CD3-positive T cell areas, reported on a 0–255 scale. Immune cell density was calculated as the percentage of CD3-, CD4-, CD8-, FOXP3-, or CD20-positive area, and macrophage content as the CD68-positive area. Histospots with <3% tissue or excessive necrosis were excluded from analysis.

Statistical analysis

All analyses were performed using JMP software (version 5.0, SAS Institute, Cary, NC, USA). PDCD4 immunofluorescence scores were evaluated as continuous variables or categorized at the median. Associations between PDCD4 expression (including subcellular localization) and tumor characteristics, metastatic site (intracranial vs. extracranial), or immune cell infiltration were assessed using the two-sample t test (or ANOVA) for continuous data and the Chi-square test for categorical variables. Prognostic relevance was determined using Cox proportional hazards models, with overall survival as the endpoint. Kaplan–Meier curves were generated to visualize survival distributions, and relationships between continuous QIF scores and clinical or pathological parameters were further analyzed using t tests or ANOVA.

Single-cell RNA sequencing of melanoma brain metastasis

A fresh melanoma brain metastasis specimen was collected during routine care with patient consent under an IRB-approved protocol at Yale. Tissue was minced and enzymatically dissociated in Hank's Balanced Salt Solution containing Collagenase IV (2.5 mg/mL) and DNAse I (0.2 mg/mL) at 37 °C for 30 minutes. Cells were separated via Lymphoprep density gradient and stained for CD45 and TcRab markers before sorting on a Becton Dickinson FACS Aria II using live/dead discrimination. Equal proportions of live CD45+/TcRab+ and live CD45+ cells were combined for downstream analysis.

Single-cell RNA libraries were prepared using the 10× Genomics platform and sequenced on an Illumina NovaSeq S4 instrument (26 × 8 × 91 bp reads; ~300 million reads per sample). Data processing was performed with Seurat v3.0: cells with <15% mitochondrial reads were retained, log-normalized, and 2000 highly variable genes were identified. After scaling and principal component analysis (15 components), dimensionality reduction and clustering were performed using UMAP and the FindNeighbors/FindClusters functions (resolution = 0.5). Cluster annotation relied on canonical lineage markers, including CD3E, CD4, CD8A (T cells), FOXP3 (Tregs), MS4A1 (B cells), KIT (mast cells), IGHG3 (plasma cells), C1QA (microglia), CD14 (macrophages), and GNLY (NK cells). Genes associated with PDCD4 expression were identified using Spearman correlation and p-values adjusted for multiple comparisons.

Results and Discussion

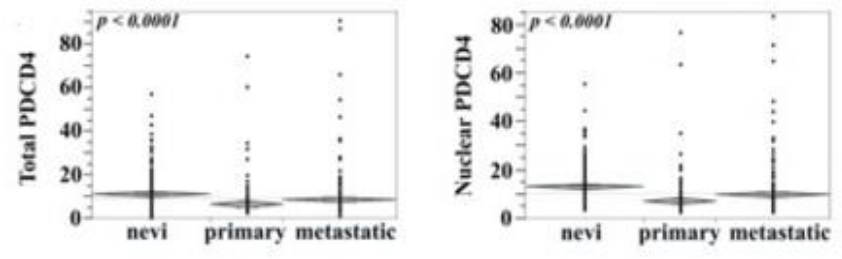
Shift from nuclear to cytoplasmic PDCD4 during melanoma progression

PDCD4's function varies depending on its localization within the nucleus or cytoplasm across multiple cancers [27, 43, 44]. While compartment-specific expression has been linked to melanoma aggressiveness in vitro, its distribution in human melanoma tissues during metastasis remains poorly characterized. Our previous observations indicated two predominant PDCD4 patterns in metastatic melanoma: combined nuclear/cytoplasmic and nuclear-only staining. In this study, PDCD4 expression was quantified in the S100-positive melanocyte/melanoma compartment as "total PDCD4" (nuclear + cytoplasmic) and in DAPI-positive nuclei as "nuclear PDCD4."

Analysis of a large cohort of 523 melanoma cases (230 primary, 293 metastases) and 263 benign lesions showed significantly higher PDCD4 expression in benign tissue compared with melanoma, irrespective of subcellular localization (ANOVA, $p < 0.0001$, **(Figure 1a)**). Dichotomizing total PDCD4 by median immunofluorescence intensity revealed that 76.0% (168/221) of nevi exhibited high expression versus 33.1% (44/133) of primary melanomas and 60.8% (127/209) of metastatic tumors (Chi-square, $p < 0.0001$). Analysis of nuclear PDCD4

yielded similar trends: 95.5% of nevi showed high nuclear expression, compared with 33.8% of primary and 60.3% of metastatic melanomas (Chi-square, $p < 0.0001$, **(Figure 1b)**).

Normal skin demonstrated strictly nuclear PDCD4 (12/12 cases, 100%), whereas nevi predominantly exhibited cytoplasmic staining. Melanomas displayed either nuclear, cytoplasmic, or both compartments **(Figure 1c)**. The proportion of nevi with cytoplasmic/nuclear staining was 83.5% (152/182), decreasing progressively in primary (68.97%, 80/116) and metastatic melanoma (63.64%, 105/165) (Chi-square, $p < 0.0001$, **(Figure 1d)**). Stratifying by predominant compartment confirmed significantly higher total and compartment-specific PDCD4 levels in metastases versus primaries (Chi-square, $p < 0.0001$ and $p = 0.0002$). Subcellular PDCD4 localization did not correlate with 5-year survival in primary or metastatic melanoma (log-rank $p = 0.47$ and $p = 0.39$).



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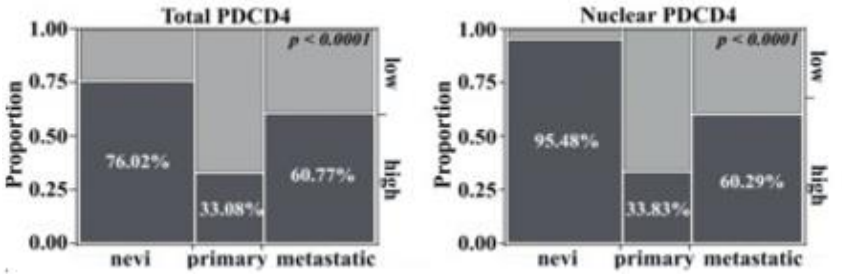

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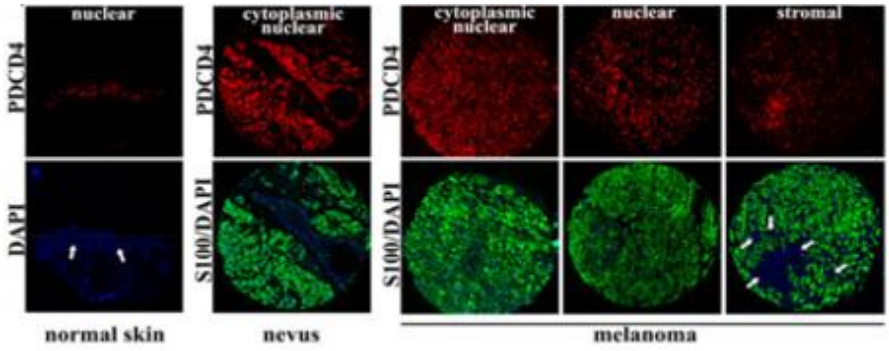

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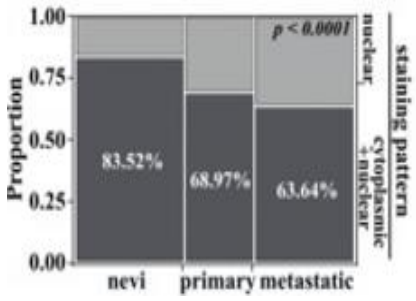


d)

Figure 1. Dynamics of PDCD4 Expression Across Melanoma Progression.

(a) Quantitative analysis revealed that PDCD4 was more abundantly expressed in benign tissue compared to malignant lesions in both the total PDCD4 (S100-positive melanocyte/melanoma compartment) and nuclear

PDCD4 (DAPI-positive nuclei) compartments (ANOVA, $p < 0.0001$). (b) High PDCD4 expression was most prevalent in nevi, decreased in primary melanomas, and rose again in metastatic melanomas, a trend observed for both total and nuclear PDCD4. (c) Representative immunofluorescence images illustrate PDCD4 (red) distribution within cytoplasm (green, S100) versus nuclei (blue) in normal skin, nevi, and melanoma. Melanoma lesions displayed three distinct PDCD4 localization patterns: cytoplasmic/nuclear, primarily nuclear, or stromal. Arrows indicate epidermis (left panel) or tumor stroma (right panel). Images were captured at 10× magnification. (d) The fraction of cells showing combined cytoplasmic and nuclear PDCD4 decreased during the transition from nevi to primary and metastatic melanoma (dark gray), whereas nuclear-only PDCD4 increased (light gray).

We also examined whether PDCD4 subcellular localization was associated with clinicopathological features. As this cohort dates from 1959 to 2000, exposure to modern immune checkpoint inhibitors was minimal, with only interferon and high-dose IL-2 used as immunotherapy. Among the small subset of treated patients (11/199 primary cases and 48/280 metastatic cases), nuclear-restricted PDCD4 localization was observed more frequently than cytoplasmic staining (Chi-square $p = 0.033$), though the small sample size limits interpretation. No other clinicopathological parameters correlated significantly with PDCD4 distribution.

Elevated PDCD4 expression predicts better 5-year survival in primary melanomas but not metastatic tumors

Previous studies have shown that while total PDCD4 levels in brain metastases were comparable to paired extracranial metastases, subcellular localization differed, with cytoplasmic PDCD4 predominating in intracranial lesions. Higher PDCD4 expression in brain metastases was linked to improved survival [22]. To further investigate its prognostic relevance, we evaluated PDCD4 expression in our extensive primary and metastatic melanoma TMA cohort. Immunofluorescence scores were categorized into high and low groups based on the median. Kaplan–Meier survival analysis revealed that patients with high total PDCD4 expression in primary melanomas had significantly better 5-year survival outcomes (**(Figure 2a)**; log-rank $p = 0.038$; RR 0.704; 95% CI 0.491–0.97; $p = 0.031$). High nuclear PDCD4 also showed a trend toward improved survival in primary tumors (**(Figure 2b)**; log-rank $p = 0.085$; RR 0.914; 95% CI 0.803–0.998; $p = 0.043$), although the effect was less pronounced.

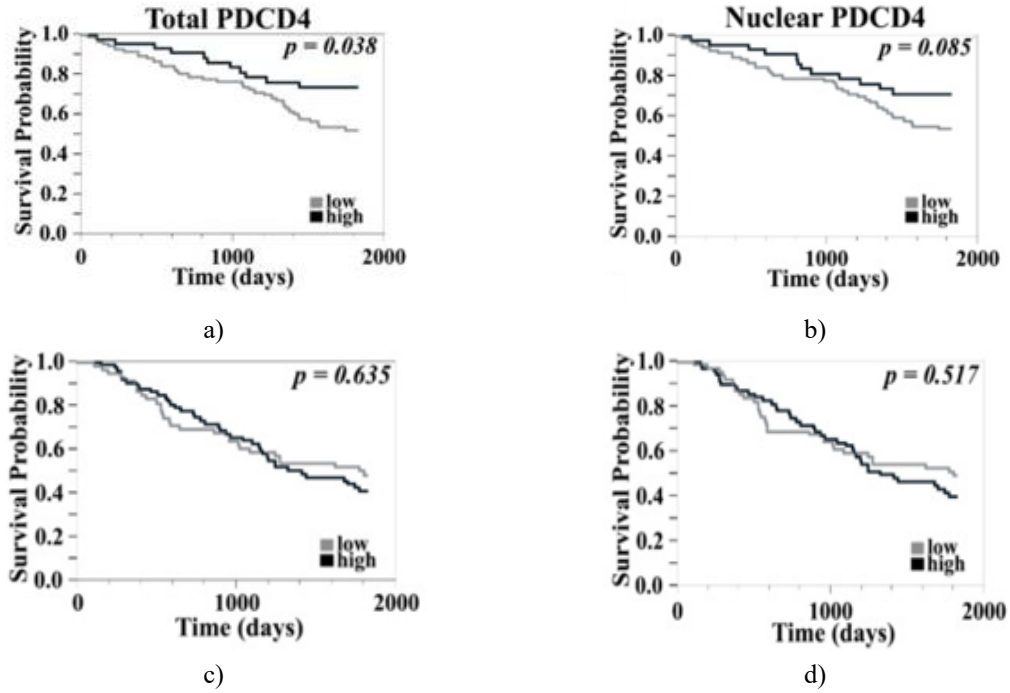


Figure 2. PDCD4 Expression and 5-Year Survival in Melanoma.

(a) High overall PDCD4 levels in primary melanomas were linked to better 5-year survival. (b) Elevated nuclear PDCD4 in primary tumors showed a positive trend, though it did not reach statistical significance. In

metastatic melanomas, PDCD4 abundance—whether measured across the entire tumor or in the nucleus—was not associated with 5-year survival outcomes (c, d).

In line with previous observations from smaller matched cohorts of intracranial and extracranial lesions, PDCD4 expression did not appear to influence survival in metastatic melanoma (log-rank p = 0.63 and p = 0.52 for total and nuclear PDCD4, respectively).

PDCD4 levels in primary tumors are linked to clark stage and absence of microscopic satellites

Given the survival advantage observed with higher PDCD4 in primary melanomas, we assessed its association with other tumor characteristics. In primary lesions, increased total and nuclear PDCD4 were significantly associated with higher Clark levels (p = 0.010 and 0.011, respectively) and the absence of microscopic satellite lesions (p = 0.0093 and 0.010). No correlation was found with Breslow thickness, ulceration status, patient age, sex, or prior immunotherapy exposure.

PDCD4 expression in stromal cells correlates with survival and immune infiltration

PDCD4 was detected not only in tumor cells (cytoplasmic and/or nuclear) but also in stromal immune cells. We evaluated PDCD4 in the stromal compartment of both primary and metastatic melanomas, its relationship with tumor PDCD4, and its impact on survival. Metastatic tumors exhibited higher stromal PDCD4 compared with primary melanomas (p = 0.029). Expression in the stroma strongly correlated with tumor cell PDCD4 in both primary ($R^2 = 0.60$, $p < 0.0001$) and metastatic samples ($R^2 = 0.73$, $p < 0.0001$).

In primary melanomas, high stromal PDCD4 was associated with improved 5-year survival (log-rank p = 0.045; RR 0.738; 95% CI 0.54–0.98; p = 0.042). Conversely, in metastatic melanomas, no survival benefit was observed (log-rank p = 0.70). Stromal PDCD4 levels were not associated with Breslow thickness, Clark stage, ulceration, patient age, sex, or prior immune therapy; however, tumors without microsatellites had higher stromal PDCD4 (p = 0.025). High stromal PDCD4 in primary lesions tended to coincide with brisk/diffuse tumor-infiltrating lymphocytes (p = 0.132), while in metastatic tumors, it was significantly associated with increased TIL density (p = 0.003). These findings suggest that PDCD4 expression in stromal cells, particularly T cells, may play a role in anti-tumor immunity in melanoma.

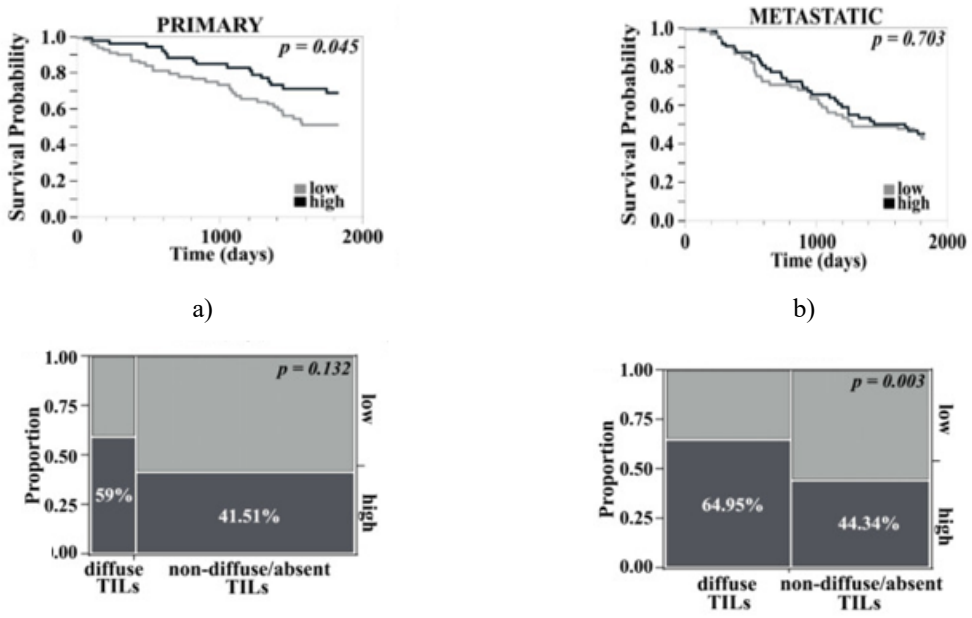


Figure 3. Stromal PDCD4 Expression and Its Relationship with Survival and Immune Infiltration in Melanoma.

(a) Primary melanomas with elevated PDCD4 levels in the stromal compartment were associated with improved 5-year survival.

(b) In contrast, high stromal PDCD4 in metastatic melanoma did not correlate with survival.

(c) Stromal PDCD4 was categorized as high or low and compared with the abundance of tumor-infiltrating leukocytes (TILs; brisk/diffuse vs. non-diffuse/absent). In primary tumors, there was a trend toward higher stromal PDCD4 levels being associated with brisk/diffuse TILs ($p = 0.13$).
(d) For metastatic lesions, high stromal PDCD4 strongly correlated with the presence of diffuse TILs ($p = 0.0031$).

We further analyzed a TMA containing 37 paired extracranial and intracranial melanoma metastases. PDCD4 was co-stained with CD3 to specifically evaluate its expression in T cells. Consistent with previous findings, PDCD4 levels in brain metastases were comparable to extracranial metastases, both within the total tissue area (DAPI-defined) and in the CD3-positive compartment (paired t-test, $p = 0.35$ and $p = 0.90$, respectively).
We then assessed correlations between stromal PDCD4 and immune cell densities, including T cells (CD3+), cytotoxic T cells (CD8+), helper T cells (CD4+), B cells (CD20+), and macrophages (CD68+). Tumors were stratified into high or low immune cell infiltration based on median % area. Higher PDCD4 expression in the stroma was significantly associated with increased CD3+ T cells, CD8+ T cells, CD20+ B cells, and CD68+ macrophages across both intracranial and extracranial sites ($p = 0.002$, $p < 0.0001$, $p = 0.029$, $p = 0.038$, respectively). No significant association was observed for CD4+ T cells in either location **(Figures 4a–4e)**.

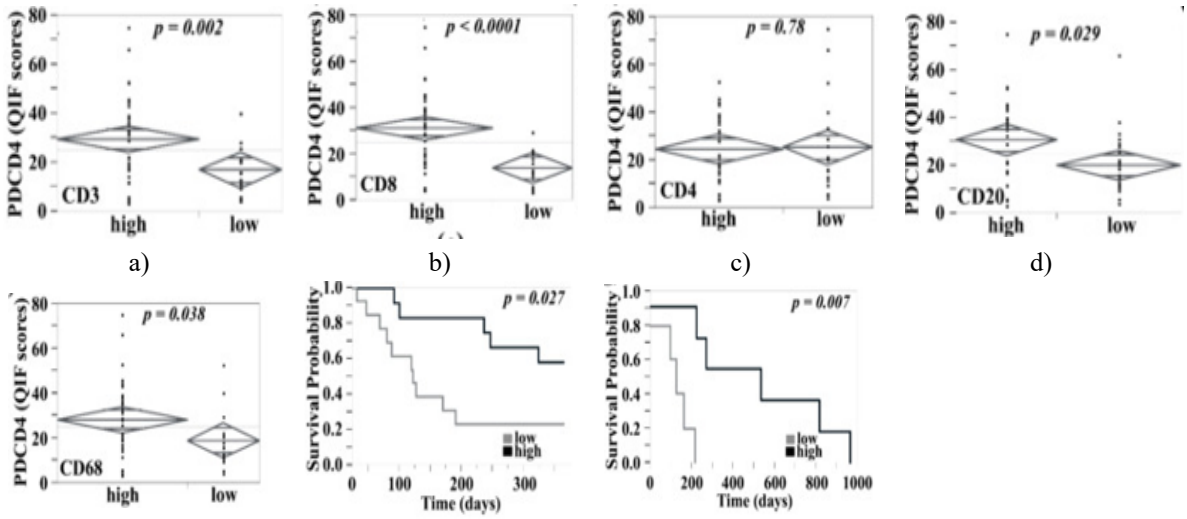
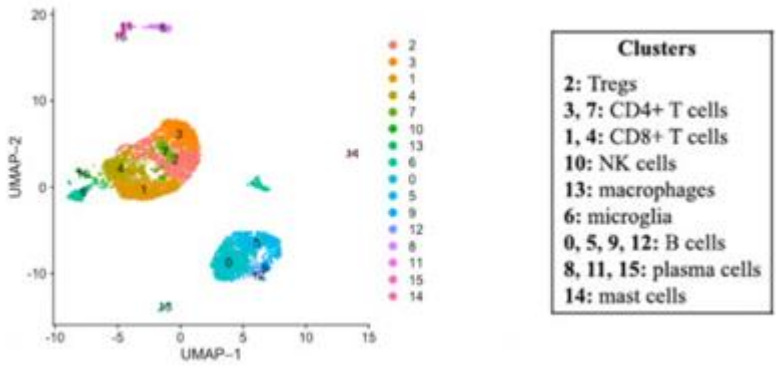


Figure 4. PDCD4 in Immune Cells and Its Impact on Survival

T cell populations in metastatic melanoma samples were divided into high and low abundance groups based on CD3+, CD8+, and CD4+ staining. Elevated stromal PDCD4 levels were observed in tumors with higher CD3+ and CD8+ T cell content ($p = 0.002$ and $p < 0.0001$, respectively), whereas CD4+ T cell abundance did not show a significant correlation. B cell density, measured by CD20+ staining, was positively associated with stromal PDCD4 ($p = 0.029$), and similarly, tumors with greater macrophage content (CD68+) also exhibited higher PDCD4 expression ($p = 0.038$).
Assessing the link between PDCD4 and patient outcomes, higher total tumor PDCD4 expression corresponded to improved 1-year survival following brain metastasis diagnosis ($p = 0.024$). Additionally, high PDCD4 levels specifically in CD3+ T cells within intracranial metastases were associated with longer intervals before brain metastasis development (brain metastasis-free survival, $p = 0.007$).
Analysis of paired tumor and stroma compartments showed that elevated PDCD4, whether in tumor cells or surrounding T cells, predicted better survival independently of BRAF/NRAS mutation status or exposure to modern treatments such as checkpoint inhibitors. These findings suggest that PDCD4 serves as an independent prognostic marker, although sample size limitations should be noted.

PDCD4 expression in specific immune populations
Single-cell RNA sequencing of immune cells from a resected melanoma brain metastasis revealed that PDCD4 is present not only in CD8+ T cells and NK cells but also in B cells and mast cells. In T cells, PDCD4 expression

correlated with cytotoxic markers, including high GZMK, GZMA, NKG7, and CTSW expression. PDCD4+ B cells also displayed cytotoxic-associated gene expression, particularly elevated GZMA levels. These results indicate that PDCD4 is expressed across multiple immune subsets and may reflect enhanced immune activity within the tumor microenvironment.



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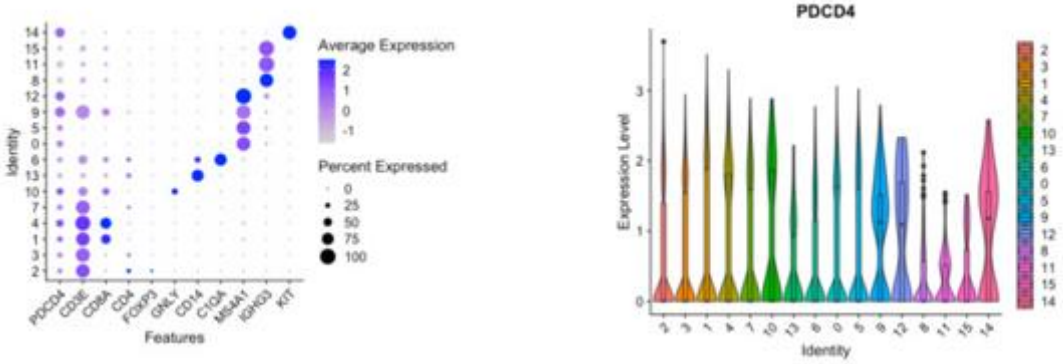

b) c)

Figure 5. Single-cell sequencing reveals PDCD4 expression on cytotoxic immune cells. (**a**) Uniform ManifoldApproximation and Projection (UMAP) image displaying clustered cell populations. (**b**) PDCD4 expression is highest in CD8+ T cells (clusters 1 and 4 based on CD3E and CD8A expression), B cells (clusters 0, 5, and 12 based on MS4A1 expression), NK cells (cluster 10 based on GNLY expression), and mast cells (cluster 14 based on KIT expression). Of note, cluster 9 expressed genes which overlap with CD8+ T cells and B cells based on expression of CD3E, CD8A, and MS4A1. (**c**) PDCD4 expression level by cell cluster.

The precise role of PDCD4 in cancer development and progression remains incompletely understood. Prior investigations into PDCD4 in human melanoma are limited, often focusing on cell lines without correlating clinical outcomes [45]. In this study, we provide a detailed assessment of PDCD4 subcellular localization throughout melanoma progression, comparing extracranial and intracranial metastases. Our work is distinct in several ways: (1) we applied automated, quantitative methods to measure PDCD4 expression in clinical specimens, reducing the subjectivity inherent in traditional pathologist-based immunohistochemistry; (2) we examined the prognostic relevance of PDCD4 in relation to its nuclear versus cytoplasmic localization; and (3) we evaluated PDCD4 expression in both tumor and stromal immune compartments.

PDCD4 is an RNA-binding protein involved in RNA metabolism and translation, and its localization is dynamically regulated, including Crm-1-dependent nuclear export under stress conditions [43]. Prior studies reported reduced PDCD4 mRNA in melanomas compared to adjacent normal tissue but did not distinguish between nuclear and cytoplasmic compartments. Functional studies indicate that PDCD4 overexpression in melanoma cells reduces proliferation, promotes apoptosis, and limits invasive potential [46], consistent with our previous observation that higher PDCD4 levels inversely correlate with tumor proliferation. MicroRNAs, such as miR-21, transcriptionally repress PDCD4 and other tumor suppressors (e.g., PTEN), promoting tumor growth and

metastasis in preclinical melanoma models [47]. Importantly, PDCD4 is also regulated at translational and post-translational levels, highlighting why protein measurements, rather than mRNA, are more indicative of its functional significance. This aligns with our prior work, which identified post-translational PDCD4 degradation mediated by the crosstalk between PLEKHA5, PI3K/AKT signaling, and the ubiquitin-proteasome pathway in brain-tropic melanomas [22].

The mechanisms controlling PDCD4 localization and its interaction with PLEKHA5 remain under investigation. PDCD4 trafficking is context-dependent, influenced by PI3K/AKT/P70S6K and RAS/MAPK signaling [48–50]. Studies in colon cancer demonstrate an inverse correlation between nuclear PDCD4 and phosphorylated AKT, and that increased nuclear and cytoplasmic pAKT is linked to cytoplasmic translocation of PDCD4 [51]. In melanoma brain metastases, PI3K/AKT activity is elevated compared to matched extracranial tumors, whereas MAPK signaling is largely unchanged [52, 53]. This suggests that intracranial microenvironmental cues may drive PDCD4 relocation from the nucleus to the cytoplasm, emphasizing the need for further research on PI3K/AKT dynamics and PLEKHA5 interactions in cerebral versus extracranial metastases.

While total PDCD4 expression declines from nevi to melanoma, our findings suggest that the shift from nuclear to cytoplasmic localization is more clinically relevant. PDCD4 is confined to the nucleus in normal skin but becomes predominantly cytoplasmic in nevi, primary tumors, and metastatic melanomas, reflecting disease progression. In cerebral metastases, PDCD4 is largely cytoplasmic, reinforcing prior observations that nuclear-to-cytoplasmic translocation correlates with aggressive disease [27, 54]. Similar localization shifts have been reported in colon cancer [55], suggesting that cytoplasmic PDCD4 may be a marker of tumor progression across malignancies. While overall staining intensity modestly increased from primary to metastatic tumors, the distinction between nuclear and cytoplasmic localization was crucial for identifying differences. In primary melanomas, higher PDCD4 levels were associated with increased Clark level and the absence of microscopic satellites, potentially reflecting a role in restraining local tumor aggressiveness. Notably, PDCD4 expression was highest in benign nevi, supporting its potential function as a barrier to malignant transformation.

Our prior work indicated an inverse relationship between PLEKHA5 and PDCD4: PLEKHA5 positively correlated with proliferation (Ki-67), whereas PDCD4 negatively correlated with tumor growth. In the present study, high PDCD4 expression in primary melanomas was associated with improved 5-year survival, but this association was not observed in metastatic tumors, most of which were extracranial. Additionally, elevated PDCD4 correlated with improved metastasis-free survival, underscoring its potential as a prognostic biomarker. Given its role in modulating tumor growth and enhancing sensitivity to chemotherapeutics and radiation [24, 31–34, 56], PDCD4 may also have predictive utility, meriting further investigation in melanoma management.

We explored PDCD4 expression in immune cells within the tumor stroma, an area that remains poorly characterized in melanoma. Previous studies showed PDCD4 on CD4+ [57] and CD8+ T cells, as well as NK cells [58]. In mouse models of hyperlipidemia, lack of PDCD4 reduced CD8+ T cells and increased regulatory T cells [59]. Using single-cell RNA sequencing from a melanoma brain metastasis, we detected PDCD4 not only in CD8+ T cells and NK cells but also on B cells and mast cells—cell types not previously reported to express this protein. Although mast cells are rare in normal brain tissue, our selection for CD45+ immune cells allowed their detection, suggesting these populations may expand in pathological states. PDCD4 was higher in CD8+ than CD4+ T cells, and its expression correlated with genes related to cytotoxicity, suggesting a role in immune-mediated tumor cell killing, which may contribute to improved survival in patients with brain metastases. Although QIF showed an association with CD68+ macrophages, single-cell data revealed low PDCD4 in macrophages (CD14+/C1QA−) and microglia (C1QA+) in this sample.

Stromal PDCD4 was elevated in metastatic melanomas compared to primary tumors, but this increase did not correlate with improved survival. In contrast, in primary tumors, higher stromal PDCD4 was linked to better 5-year survival. In brain metastases, elevated PDCD4 in the total tissue compartment also predicted longer survival. The presence of PDCD4 in cytotoxic CD8+ T cells may explain the survival benefit observed in primary melanomas. Future work is needed to identify which immune subsets drive these outcomes and to clarify the functional roles of PDCD4 in B cells and mast cells. Additionally, miR-21 negatively regulates T cell activation and memory, and its loss can increase PLEKHA1 expression [60]. Given the link between PLEKHA1 and PLEKHA5, it is worth investigating whether miR-21 also affects PLEKHA5 and its interaction with PDCD4 in immune cells.

It is important to note that our samples were collected before modern checkpoint inhibitors were widely used, and some patients received older therapies such as interferon or high-dose IL-2. Therefore, the predictive value of

PDCD4 in the context of contemporary immunotherapy requires further study. Moreover, because our TMA did not include matched primary and metastatic samples from the same individuals, changes in PDCD4 expression over the course of disease or treatment remain unclear.

Conclusion

PDCD4 appears to play a role early in neoplastic transformation, with peak expression in benign nevi. During melanoma progression, PDCD4 increases again in metastatic lesions and shifts from nuclear to cytoplasmic localization. Elevated PDCD4 in tumor and stroma is linked to better survival in primary melanomas and brain metastases, but not in extracranial metastases. The survival benefit in brain lesions appears independent of PDCD4's subcellular location or stromal expression.

We also confirmed PDCD4 expression in key cytotoxic immune cells, including CD8+ T cells and NK cells, and identified novel expression in B cells and mast cells. PDCD4 expression correlates between tumor and stromal compartments and is associated with higher immune infiltration. Notably, higher PDCD4 in CD3+ T cells predicted improved brain metastasis-free survival. These findings suggest PDCD4 may serve as a prognostic biomarker for intracranial disease and may offer new therapeutic opportunities for enhancing anti-tumor immunity in melanoma.

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