The prognostic and immunological impacts of DCX expression: a pan-cancer analysis

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**Author contributions**
Li WR performed data analysis and original draft writing; Li X and Jin H performed original draft writing; Li X and Jin H reviewed and edited the manuscript; Li X and Jin H performed the study concept and design; Li X and Jin H is the guarantor of this work and takes responsibility for the integrity of the data and accuracy of the data analysis. All the authors have read and approved the final manuscript.

**Competing interests**
The authors declare no conflicts of interest.

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**Abbreviations**
DCX, doublecortin; TME, tumor microenvironment; DESeq2, differential expression sequencing 2; TMB, tumor mutational burden; MSI, microsatellite instability; ACC, adenocortical carcinoma; LGG, low-grade glioma; LHC, liver hepatocellular carcinoma; LADC, lung adenocarcinoma.

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**Abstract**
**Background:** Doublecortin (DCX), a microtubule-associated protein, is best known for its critical role in neuronal migration during neural development, where it stabilizes microtubules and guides neurons to their proper positions. Recently, DCX has been implicated in various cancer processes, suggesting it may influence tumor progression and the tumor microenvironment. Emerging evidence indicates that DCX can modulate cell migration, invasion, and interaction with immune cells, making it a potential player in oncogenesis. However, the role of DCX across different cancer types and its potential as a prognostic biomarker remain underexplored, necessitating a comprehensive analysis. **Methods:** We utilized The Cancer Genome Atlas to extract data on DCX expression in tumor and adjacent normal tissues across diverse cancer types. Differential expression analysis was conducted using differential expression sequencing 2. Survival analysis was performed with Kaplan-Meier estimates and Cox proportional hazards models. Correlations between DCX expression and tumor mutational burden, microsatellite instability, and immune infiltration were examined using Spearman’s correlation. **Results:** DCX showed variable expression across cancer types, with significant overexpression in certain tumors such as liver and lung cancer and downexpression in others like breast cancer. High DCX expression was correlated with poor prognosis in adenocortical carcinoma but with better outcomes in low-grade glioma. Additionally, DCX expression was significantly associated with various immune markers and chemokines, suggesting a role in modulating the immune microenvironment. **Conclusion:** Our findings highlight the complex role of DCX in cancer, underlining its potential as a prognostic marker and its involvement in immune-related pathways. Targeting DCX could represent a novel approach to modulating tumor behavior and enhancing immune response in cancer therapy. **Keywords:** pan-cancer analysis; tumor microenvironment; prognostic biomarker; immune infiltration; chemokine signaling; cancer genomics
Background
Cancer is a complex disease characterized by the aberrant expression of genes that regulate cell growth, differentiation, and interactions within the immune system. Among these, doublecortin (DCX), a microtubule-associated protein traditionally known for its role in neuronal migration, has recently emerged as a gene of interest in oncology. While its expression and functions are well-documented in neurological development, the implications of DCX expression in various cancers remain less explored. Emerging studies suggest potential roles for DCX in tumorogenesis, metastasis, and modulation of the tumor microenvironment (TME), indicating its broader impact on cancer progression and patient outcomes [1–3].

The TME, pivotal in cancer progression and response to therapy, is significantly influenced by the interaction between tumor cells and the host's immune system. Chemokines and their receptors play vital roles in this interplay, affecting immune surveillance and the inflammatory response. Furthermore, the expression of immune checkpoints, which can suppress the anti-tumor immune response, and tumor mutational burden (TMB) are critical factors that shape responsiveness to immunotherapies[4–6]. Given the complexity of these interactions, understanding the molecular drivers that influence these pathways is crucial for the development of targeted therapies.

This study delves into the expression of DCX across a spectrum of cancers, examining its association with prognostic markers such as TMB and microsatellite instability (MSI), its influence on immune infiltration, and its correlation with chemokine signaling. By investigating the differential expression of DCX between tumor and normal tissues, as well as its association with patient survival and immune modulation, this research aims to elucidate the potential of DCX as a biomarker for cancer prognosis and a therapeutic target [7].

Understanding the multifaceted role of DCX in cancer could not only enhance our comprehension of tumor biology but also pave the way for novel therapeutic strategies. These strategies might exploit DCX expression to manipulate the TME, potentially improving the efficacy of existing treatments.

Methods
Data collection and preprocessing
Gene expression data for DCX across various cancer types were obtained from the Cancer Genome Atlas. This dataset includes both tumor and adjacent normal tissue samples from multiple cancer types. Raw data were normalized using the Reads Per Kilobase Million method to account for differences in gene length and sequencing depth.

Differential expression analysis
Differential expression of DCX between tumor and normal tissues was analyzed using the differential expression sequencing 2 (DESeq2) R package. This method utilizes a model based on the negative binomial distribution. Significance was set at P < 0.05.

Survival analysis
Survival data were analyzed to investigate the prognostic impact of DCX expression. Patients were stratified into high and low expression groups based on the median expression level of DCX. Survival curves were generated using the Kaplan-Meier method, and differences in survival outcomes were assessed using the log-rank test. Cox proportional hazards regression was used to calculate hazard ratios and 95% confidence intervals.

Correlation with immune markers
Correlation analyses between DCX expression and TMB, MSI, and immune infiltration scores were conducted using Spearman’s rank correlation coefficient. Immune infiltration scores were obtained using the estimation of stromal and immune cells in malignant tumor tissues using expression data algorithm to derive stromal and immune scores from the gene expression data.

Immunofluorescence and immunohistochemistry
Immunofluorescence staining of DCX was performed on 4 µm thick formalin-fixed, paraffin-embedded tissue sections. Sections were deparaffinized, rehydrated, and antigen retrieval was performed using citrate buffer (pH 6.0) at high temperature. Sections were then blocked and incubated with anti-DCX primary antibody followed by a fluorescently labeled secondary antibody. Nuclei were stained with 4'-6-diamidino-2-phenylindole, and slides were mounted with an anti-fade mounting medium.

For immunohistochemistry, sections underwent similar preparation and were incubated with a primary antibody against DCX. Detection was performed using an horseradish peroxidase-conjugated secondary antibody and 3,3'-diaminobenzidine as a chromogen. Slides were counterstained with hematoxylin to visualize nuclei.

Rationale for experiments
The rationale for conducting differential expression analysis was to identify whether DCX expression levels vary significantly between tumor and adjacent normal tissues across different cancer types. Understanding these differences can provide insights into the potential role of DCX in tumorgenesis. Survival analysis was performed to determine the prognostic significance of DCX expression, exploring whether higher or lower expression levels are associated with patient outcomes in specific cancers. This analysis helps identify cancers where DCX could serve as a prognostic biomarker.

Statistical software and tools
All statistical analyses were performed using R software (version 3.6.1). Figures were created using the ggplot2 package in R. DESeq2 was used for differential expression analysis, leveraging its robust modeling of count data based on the negative binomial distribution. This method is widely accepted for analyzing RNA-Seq data due to its ability to handle varying sequencing depths and biological variability [8]. For survival analysis, Kaplan-Meier estimates were generated and differences in survival outcomes were assessed using the log-rank test. Cox proportional hazards models were used to calculate hazard ratios and 95% confidence intervals, providing a comprehensive assessment of the prognostic impact of DCX expression. The Spearman’s rank correlation coefficient was employed to examine the relationships between DCX expression and TMB, MSI, and immune infiltration scores.

Results
Differential expression of DCX gene across various cancer types
In our pan-cancer analysis, the expression of the DCX gene was compared between several cancer types and their corresponding normal tissues (Figure 1A). The findings reveal a notable decrease in DCX expression in tumor tissues compared to normal tissues in breast cancer, while in liver hepatocellular carcinoma (LHCC), DCX expression was significantly increased in tumor tissues (P < 0.01). Additionally, we assessed cancers with matched samples, i.e., tumor tissues and corresponding non-tumor tissues from the same patients (Figure 1B). In lung cancer (LUAD), a significant difference was observed, with tumor tissues showing higher DCX expression levels compared to matched normal tissues (P < 0.05).

In addition to adult cancers, we explored the expression of DCX in pediatric tumors using the TARGET database. Our analysis revealed significant differential expression of DCX across different stages of pediatric neuroblastoma, suggesting that DCX plays a crucial role in both adult and pediatric cancers (Supplementary Figure S1)

Localization of DCX protein in cellular compartments
The localization of DCX protein within cells and tissues was investigated using immunofluorescence and immunohistochemistry techniques (Figures 1C and 1D). Immunofluorescence results indicated that DCX protein predominantly localizes in the cytoplasm, suggesting a potential role in cellular structure or function. Immunohistochemical analysis further confirmed this distribution pattern, showing
Advances using with (C) of where with blue association The labeled DCX with method log-rank expression tissue DCX = interval: ratio cancer points y-axis (LGG), a across across tissue. instance, of different interval. is TMB a infiltration better DCX was were various and adrenocortical 1 DCX Notably, cancer o across Expression expression TPM) correlations cell significance = types DCX (A) hazard and glioma outcomes. (D) 1.342 on indicate between in points TMB confidence immune of significantly tissue MSI expression DCX < different vs. cancer 2024; DCX, tissue 4’-6-diamidino-2-phenylindole Immunofluorescence in Significant poor in with expression demonstrated high progression-free the expression. significant impact patient Figure types Prognostic samples. widespread differences in normal tumor tissues, showing marked differences in normal tissue. The y-axis is labeled “DCX expression (log2 TPM)”. Significant differences are indicated by asterisks (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant). (B) Scatter plots of DCX expression in paired tumor and normal samples for selected cancers with significant expression differences. Each point represents an individual sample pair; red points indicate tumor tissue and blue points indicate normal tissue. The y-axis is labeled “DCX Expression (log2 TPM)”. (C) Immunofluorescence images showing the localization of DCX protein in tumor cells. DCX protein is visualized in green, nuclei are stained with 4’-6-diamidino-2-phenylindole (blue), and cytoskeletal actin is marked in red. Scale bar: 20 μm. (D) Immunohistochemical staining of DCX in tumor tissue sections. Brown staining indicates the presence of DCX protein, counterstained with hematoxylin (blue) to show nuclei. Scale bar: 50 μm. DCX, doublecortin.

Figure 1 Expression of DCX in cancer vs. normal tissues across multiple cancer types. (A) Box plots representing the differential expression of DCX in tumor tissues compared to matched normal tissues across a variety of cancer types. The cancers are sorted by the relative DCX expression levels between tumor and normal tissues, with significant differences highlighted in green. The y-axis is labeled “DCX expression (log2 TPM)”. Significant differences are indicated by asterisks (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant). (B) Scatter plots of DCX expression in paired tumor and normal samples for selected cancers with significant expression differences. Each point represents an individual sample pair; red points indicate tumor tissue and blue points indicate normal tissue. The y-axis is labeled “DCX Expression (log2 TPM)”. (C) Immunofluorescence images showing the localization of DCX protein in tumor cells. DCX protein is visualized in green, nuclei are stained with 4’-6-diamidino-2-phenylindole (blue), and cytoskeletal actin is marked in red. Scale bar: 20 μm. (D) Immunohistochemical staining of DCX in tumor tissue sections. Brown staining indicates the presence of DCX protein, counterstained with hematoxylin (blue) to show nuclei. Scale bar: 50 μm. DCX, doublecortin.
Figure 2 Prognostic significance of DCX expression in various cancers. (A) Forest plot summarizing hazard ratios for high versus low DCX expression across multiple cancer types with confidence intervals and p-values highlighting its prognostic impact. The x-axis is labeled “hazard ratio”. (B) Heatmap categorizing cancers into groups based on the prognostic significance of DCX expression using Cox regression and log-rank tests, with hierarchical clustering of both rows and columns. Colors indicate whether DCX is a risk factor (red), protective (green), or non-significant (grey). (C, D) Kaplan-Meier survival curves for ACC and LGG, respectively, stratifying patients by high (yellow line) and low (blue line) DCX expression. The x-axis is labeled “Time (Months)” and the y-axis is labeled “survival probability”. DCX, doublecortin; ACC, adrenocortical carcinoma; LGG, low-grade glioma; HR, hazard ratio; DFI, disease-free interval; DSS, disease-specific survival; OS, overall survival; PFI, progression-free interval.
Correlation of DCX expression with immune checkpoint and immunomodulatory gene sets

Figure 4 investigates the correlation between DCX gene expression and a diverse set of immune-related gene sets across different cancer types. Figure 4A illustrates the correlations between DCX and classical immune checkpoint molecules. Notable findings include a significant positive association between DCX and CTLA-4 in ACC, suggesting a potential role for DCX in modulating immune checkpoint pathways in this particular cancer. This association indicates that higher DCX expression might be linked with an enhanced expression of inhibitory checkpoint molecules, potentially contributing to immune evasion.

Figure 4B explores the relationships between DCX expression and immune suppressive molecules. Significant correlations were noted with TGF-beta 1 in glioblastoma and V-domain Ig suppressor of T cell activation (identified as HAVCR2) in LGG. These findings suggest that DCX may influence the TME by promoting an immune suppressive milieu, potentially impairing anti-tumor immune responses in these contexts. Finally, Figure 4C examines the correlation between DCX expression and immune activating molecules. Noteworthy is the positive association with members of the TNF superfamily, specifically TNF59F in ACC and TNF58F in colorectal cancer. These correlations suggest that DCX might also be involved in stimulating immune responses, which could either aid in anti-tumor immunity or contribute to inflammation and cancer progression.

Correlation of DCX expression with chemokine and chemokine receptor genes

Figure 5 delves into the relationships between DCX gene expression and chemokine and chemokine receptor genes across various cancers, highlighting its influence in modulating the TME. Figure 5A investigates the correlations between DCX expression and classic chemokine molecules across diverse cancers. Significant correlations were found in several cancer types, highlighting the role of DCX in influencing the chemokine-mediated signaling pathways. For instance, positive correlations with CXCL10 and CXCL11 were observed in ACC and diffuse large B-cell lymphoma, suggesting that DCX may facilitate the recruitment of immune cells to the TME, potentially enhancing the immune response against the tumor. Figure 5B explores the correlations between DCX expression and classic chemokine receptor genes. The analysis indicates complex interactions, with significant correlations noted across multiple cancer types. A notable finding is the negative correlation between DCX expression and CXCR4 in glioblastoma, which might imply a role for DCX in negatively regulating immune cell trafficking or possibly modifying the immune environment to favor tumor progression or suppression depending on the context.
Figure 4 DCX expression correlated with immune checkpoint and modulatory genes. (A) Heatmap displaying the correlation between DCX and key immune checkpoint genes across various cancers, with hierarchical clustering of columns. Significant correlations are highlighted (*P < 0.05, **P < 0.01). The color scale indicates correlation coefficients. (B, C) Heatmaps of DCX correlations with genes involved in immune suppression and activation, respectively, across different cancer types, with hierarchical clustering of columns. The color scale indicates correlation coefficients. DCX, doublecortin; ACC, adrenocortical carcinoma; LGG, low-grade glioma.

Figure 5 Interaction of DCX expression with chemokines and chemokine receptors. Heatmaps detailing the correlation of DCX expression with chemokine and chemokine receptor genes across various cancer types. Each cell in the heatmap represents the correlation coefficient, with color intensity indicating the strength and direction of the correlation (red for positive, blue for negative). Asterisks denote statistical significance (*P < 0.05, **P < 0.01). DCX, doublecortin; ACC, adrenocortical carcinoma; LGG, low-grade glioma.
Recent studies have significantly expanded our understanding of the roles that DCX plays in both neural development and cancer [9, 10]. Traditionally recognized for its function in neuronal migration, DCX has been increasingly implicated in cancer progression through its modulation of the cytoskeleton and interaction with key signaling pathways. For instance, DCX's involvement in the Wnt/β-catenin pathway, which regulates cell proliferation and differentiation, highlights its potential impact on oncogenesis [11].

Moreover, DCX expression has been associated with the TME, influencing immune cell infiltration and activity. High DCX levels have been linked to increased regulatory T cells in some cancers, which can suppress anti-tumor immune responses, while in others, it may enhance cytotoxic T cell recruitment, suggesting a complex role in immune modulation [12–14]. These findings underscore the importance of further research to elucidate the precise mechanisms and therapeutic potential of DCX in cancer.

This study has illuminated the complex roles of DCX in a range of cancers, highlighting its variable expression and association with key prognostic factors such as TMB, MSI, immune infiltration, and chemokine signaling. Our findings contribute to a nuanced understanding of DCX's dual roles in cancer, suggesting both tumor-promoting and tumor-suppressing activities depending on the tumor type and microenvironmental context.

While our study found that high DCX expression is associated with better outcomes in LGG, it is essential to examine its prognostic value in other cancer types. Recent studies have highlighted the complex role of DCX across different malignancies. For instance, in glioblastomas, high DCX expression has also been associated with improved survival, suggesting a potential protective role in these brain tumors [2]. Conversely, in ACC, our analysis indicated that high DCX levels correlate with poorer prognosis, potentially due to its role in enhancing tumor aggressiveness through increased cell migration and invasion.

In LIHC and LUAD, overexpression of DCX was found to be significantly correlated with worse patient outcomes, indicating that DCX may promote tumor progression in these cancers. The differential impact of DCX expression on prognosis across various cancers underscores the context-dependent nature of its function. In certain cancers, DCX may contribute to maintaining cellular stability and preventing aggressive phenotypes, while in others, it may facilitate tumor progression through mechanisms such as cytoskeletal reorganization and modulation of the TME. Further studies are warranted to explore these context-specific roles of DCX in cancer.

Understanding the molecular mechanisms underlying these differences will be crucial for developing targeted therapeutic strategies that can exploit DCX expression to improve patient outcomes. Additionally, clinical validation through larger, multicenter studies could help solidify DCX's role as a prognostic biomarker and therapeutic target across a broader spectrum of cancers.

The expression trends of DCX across different cancer types suggest it may be involved in diverse biological processes depending on the cancer subtype and specific somatic genotypes. For example, in breast cancer, DCX expression is significantly downregulated in tumor tissues compared to normal tissues, which could be associated with specific subtypes such as triple-negative breast cancer that often exhibit more aggressive phenotypes. In contrast, DCX is upregulated in LIHC and LUAD, where it might be linked to particular genetic alterations or oncogenic pathways active in these cancers.

Investigating the associations between DCX expression and specific somatic genotypes, such as mutations in key oncogenes or tumor suppressor genes, could provide insights into the molecular mechanisms underlying its differential expression. For instance, in gliomas, mutations in IDH1/2 and 1p/19q co-deletions are known to define distinct subtypes with varying prognoses, and it would be interesting to explore if DCX expression correlates with these genotypic alterations.

The significant correlations between DCX expression and both chemokine and chemokine receptor gene expressions highlight its potential influence on the TME. DCX may modulate the TME by influencing chemokine-driven recruitment of immune cells. For instance, the positive correlation between DCX and CCR5 in colorectal cancer may indicate a role for DCX in attracting regulatory T cells, thus promoting an immunosuppressive environment conducive to tumor growth. Conversely, the negative correlation with CXCR4 in gliomas suggests a potential suppressive effect on the recruitment of effector immune cells, altering the balance between tumor suppression and promotion. From a therapeutic perspective, targeting DCX could modify the immune landscape of tumors, enhancing the efficacy of existing treatments, especially immunotherapies. By altering DCX expression, it might be possible to shift the immune milieu from a suppressive to a more active state, potentially increasing the tumor's vulnerability to immune attack [15–17].

Our study suggests the potential of DCX as a biomarker for cancer prognosis. Further research, including unbiased whole-genome approaches and functional studies, is necessary to validate these findings and explore the therapeutic potential of targeting DCX in various cancers. Developing drugs that modulate DCX activity could provide dual benefits, such as suppressing tumor growth in cancers where DCX is upregulated and enhancing protective roles where it is downregulated.

Moreover, since DCX influences chemokine signaling, targeting pathways that interact with DCX might yield synergistic effects. These strategies could include using chemokine receptor antagonists or agonists in conjunction with DCX modulation to reshape the immune infiltration and activity within the TME [18, 19].

To establish DCX as a viable therapeutic target, additional evidence is needed. This includes functional studies to validate its role in tumor progression and modulation of the immune microenvironment. Experiments such as CRISPR-Cas9 mediated knockdown or overexpression of DCX in various cancer cell lines, followed by assessments of changes in cell migration, invasion, and immune cell interactions, would provide more direct evidence of DCX's functional roles. Additionally, in vivo studies using animal models to observe the effects of DCX modulation on tumor growth and immune response would be crucial. Understanding how DCX's known functions in neuronal migration link to its roles in cancer is another critical area for exploration. DCX stabilizes microtubules and guides neuronal positioning, suggesting that in cancer, it might similarly influence cell structure and movement. This could explain its involvement in cell migration and invasion observed in tumors. Moreover, its interactions with the immune microenvironment might be related to its effects on cellular pathways that control immune cell recruitment and activity.

While our study provides significant insights into the role of DCX in various cancers, we acknowledge the need for further experimental validation to substantiate our findings. Due to current constraints in laboratory resources and funding, we are unable to perform these additional experiments at this time. However, we propose several future directions for research that could validate our conclusions. In vitro studies could investigate the effects of DCX overexpression and knockdown in cancer cell lines to observe changes in cell proliferation, migration, and invasion. These studies would elucidate the direct role of DCX in cancer cell behavior. Additionally, in vivo studies using animal models could help to study the impact of DCX modulation on tumor growth and metastasis, providing a comprehensive understanding of DCX's role in the TME and its interactions with the immune system. Furthermore, clinical validation through retrospective and prospective studies using patient samples could confirm the prognostic value of DCX expression in different cancer types. Collaborating with other research institutions to access a larger and more diverse dataset would enhance the robustness of these findings.

In conclusion, while our findings have positioned DCX as a potentially significant factor in cancer biology, further studies are essential to confirm its role and explore its utility as a biomarker and therapeutic target. Additional functional and in vivo studies are needed to validate DCX's involvement in tumor progression and
immune modulation. Understanding the molecular interactions and effects of DCX in cancer will be crucial for developing more effective and targeted therapeutic strategies. Our study suggests the potential of DCX as a biomarker for cancer prognosis, but its therapeutic applications require more extensive investigation.

References