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Single-cell transcriptomic profiling of malignant peripheral nerve sheath tumors

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Abstract

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas arising from Schwann cells and characterized by marked cellular and molecular heterogeneity. Although bulk multi-omic studies have provided valuable insights into MPNST biology, recent advances in single-cell profiling have deepened our understanding of the tumor microenvironment and molecular mechanisms underlying malignant transformation. Single cell analyses have revealed distinct Schwann cell-like, malignant neural crest-like, immune, and stromal cellular subpopulations within MPNSTs and their precursor lesions. Comparative profiling of MPNSTs, neurofibromas, and atypical neurofibromatous neoplasms of uncertain biologic potential, suggest that MPNST progression involves Schwann cell dedifferentiation into a more primitive, stem-like state. In this review, we summarize key discoveries from single-cell characterization studies, and discuss how these findings illuminate MPNST tumorigenesis, cellular plasticity, and potential therapeutic vulnerabilities.

Key Points

- Single-cell profiling maps malignant, immune, and stromal cell diversity in MPNSTs
- MPNSTs progression imitates Schwann-cell dedifferentiation toward primitive cell states
- PRC2 loss is associated with an aggressive, immune-cold MPNST subgroup

Malignant peripheral nerve sheath tumors (MPNSTs) are rare, aggressive soft-tissue sarcomas that can arise sporadically, from precursor lesions in patients with neurofibromatosis type 1 (NF1), or rarely, post-radiation. The lifetime risk of developing MPNST in patients with NF1 is 8-13%,[1,2] and they confer high morbidity and mortality, with the 5-year survival rate estimated at 26-39%, even with aggressive treatment.[3] The current standard of care consists of aggressive surgery with wide margins when feasible, though this can be limited by the tumor size, location, and surrounding anatomy.[3] Evidence supporting radiotherapy and systemic treatments has been contradictory and limited, in part due to the rarity of MPNSTs, studies that are small and retrospective, and pooling of MPNSTs with other biologically distinct sarcomas in clinical trials. Further complicating this challenge is the significant intratumoral and intertumoral heterogeneity of MPNSTs, which may obscure therapeutic response in specific subgroups of MPNSTs.

In patients with NF1, MPNSTs arise through malignant transformation of plexiform neurofibromas, benign nerve sheath tumors that are pathognomonic of NF1. Over the past decade, the entity "atypical neurofibromatous neoplasm with uncertain biological potential" (ANNUBP) has been identified as an intermediate stage between neurofibromas and MPNSTs. ANNUBPs are histologically defined by at least two atypical features, including hypercellularity, cellular atypia, loss of neurofibroma architecture, or increased mitotic activity.[4,5] While the loss of *NF1* appears to be sufficient for the development of neurofibromas, progression to MPNST is associated with further loss of *CDKN2A*, *TP53*, *SUZ12*, and/or *EED*.[5–9] *NF1* loss and *CDKN2A* loss are the only recurrent alterations in ANNUBPs, suggesting that *CDKN2A* loss occurs early in malignant transformation.[5,10] Compared to other cancers, the burden of mutation in MPNSTs is relatively low.[5] Instead, epigenetic dysregulation has emerged as a key driver of MPNST pathogenesis.

A well-established mechanism of epigenetic dysregulation in MPNSTs involves recurrent inactivating mutations in *SUZ12* and *EED*, components of polycomb repressor complex 2 (PRC2), which have been identified in 70-92% of MPNSTs.[11] PRC2 is a

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chromatin regulatory enzyme that mediates repression of gene expression via H3K27 methyltransferase activity, and both loss-of-function and gain-of-function mutations affecting components of PRC2 have been associated with various cancers.[12] In MPNST, PRC2 loss is a driver involved in invasiveness, metastases, immune suppression, and treatment resistance, underscoring a critical role for epigenetic dysregulation in the pathogenesis of MPNSTs.[79,10,13]

Single-cell RNA sequencing (scRNA-seq), which enables genome-wide transcriptomic characterization of individual cells or nuclei, has transformed the ability to dissect the cellular and molecular complexity of MPNSTs. Whereas bulk transcriptomic approaches average gene expression across mixed cell populations and therefore can obscure the contribution of different cell types, scRNA-seq captures the diversity of cell phenotypes in a tumor. Distinct cell states, lineage relationships, and interactions within the tumor microenvironment can be analyzed. Complementary to this, emerging spatial transcriptomic technologies measure gene expression while preserving the spatial organization of the tissue, thereby providing histologic or microenvironmental context. These techniques have contributed important findings to other cancers but has only recently been applied to studies of peripheral nerve sheath tumors.

The complex tumor microenvironment of MPNSTs, lack of targetable mutations, and bulk multi-omic studies suggestive of heterogeneity make MPNSTs particularly suited for studying at a single-cell resolution. Here, we review these single-cell studies on MPNSTs and their precursor lesions, highlighting discoveries related to intratumoral heterogeneity, molecular classification, malignant transformation, and therapeutic vulnerabilities.

Intratumoral Heterogeneity and the Tumor Microenvironment

MPNSTs and the plexiform neurofibromas they arise from are notoriously heterogeneous. Multi-regional sampling has demonstrated distinct bulk transcriptomic and copy number profiles in different areas of MPNSTs.[5,14,15] ScRNA-seq has also characterized the variety of cell types both neurofibromas and MPNSTs are comprised of. Compared to neurofibromas, MPNSTs are enriched for proliferating tumor cells, immature Schwann cells and Schwann cell precursors, and activated fibroblasts.[5,15–17] Distinct populations of fibroblasts have also been identified in nerve sheath tumors. Endoneurial fibroblasts contained within nerve fascicles, perineurial fibroblasts within the nerve fascicles, and epineurial fibroblasts outside of the nerve fascicles, were observed in a single-cell dataset of plexiform neurofibromas and distinguished by unique gene markers.[18] Aberrant Schwann cell and endoneurial fibroblasts infiltration into the epineurial zone was also observed on spatial transcriptomics of plexiform neurofibromas, indicating disrupted nerve architecture.[18] Compared to neurofibromas, MPNSTs exhibited depletion of these resident fibroblasts.[19] This structural reorganization underscores the breakdown of the typical nerve microenvironment during tumor progression. Further work is needed to understand the cancer-associated fibroblast (CAF) population in MPNSTs and assess whether CAF-directed therapies that have been promising in other cancers,[20] have potential in MPNSTs.

Single-cell profiling has also demonstrated the contribution of clonal evolution to MPNSTs heterogeneity using chromosomal copy number variations inferred from scRNA-seq. Sequencing of human surgical samples and patient-derived xenograft (PDX) models have shown that MPNSTs overall had a higher degree of aneuploidy compared to plexiform neurofibromas.[5,21] One study showed that chromosome 8q gain specifically was present in >90% of cells in MPNST PDX samples but absent in plexiform neurofibromas. Gain of chromosome 8q therefore appeared to be an early and recurrent copy number variation in MPNSTs. This same study also identified intra-tumoral CNV-defined subclones, noting that while 8q gain was detected in the vast majority of cells, other chromosomal alterations occurred in distinct subpopulations, underscoring intratumoral heterogeneity as a key feature of MPNST biology.[21]

Beyond defining cell type and composition, scRNA-seq also allows profiling of cell-cell communication within the tumor microenvironment. This approach infers potential intercellular signaling events by examining co-expression of ligand and receptor gene pairs across different cell populations.[22] High expression of both a ligand and its corresponding receptor is interpreted as evidence of potential cellular cross-talk. Wu et al. found that the strongest interaction in plexiform neurofibromas was between neoplastic Schwann cells and fibroblasts. In MPNSTs, the number of interactions was further increased (69 vs. 24 in plexiform neurofibromas), suggesting increased complexity of intercellular signaling and a more prominent role of the tumor microenvironment in malignant progression.[19]

Finally, histologically distinct regions of more benign tumor and regions of malignancy can often be observed on clinical pathology samples. Bulk transcriptomic analysis of these spatially defined regions have revealed that neurofibroma-like regions adjacent to MPNSTs are distinct from neurofibromas and ANNUBPs that are not associated with malignant transformation.[23] Upregulation of IL6, CXCL8, and HLA-DQB1 in neurofibromas regions adjacent to MPNST suggests that activation of inflammatory and immune-response pathways may contribute to malignant transformation in MPNST.[23] Future studies using emerging spatial transcriptomic technologies will enable these interactions to be examined at near single-cell resolution rather than across bulk regions, linking gene expression directly to histologic and microenvironmental context.

Collectively, single-cell studies have revealed that MPNSTs are composed of diverse malignant, stromal, and immune components engaged in a complex microenvironment. Understanding how these interactions shape tumor growth, immune evasion, and therapeutic resistance may help uncover new avenues for microenvironment-targeted therapies in MPNSTs.

Molecular and Immune Subgroups of MPNSTs

Our molecular understanding of intertumoral heterogeneity MPNSTs has been primarily derived from bulk multi-omic analyses of MPNSTs and their precursors. Epigenetic and transcriptomic profiling have revealed two stable subgroups of MPNSTs.[5,15] One group of MPNSTs ("MPNST-G1/group 1") confer significantly poorer outcomes and are enriched

for alterations in *SUZ12* and *EED*. This molecular subgroup exhibits increased chromosomal alterations, CpG island methylation, and upregulation of the sonic hedgehog pathway. In contrast, a distinct second group of MPNSTs, termed "MPNST-G2" or "group 2", is enriched with immune cells and exhibits upregulation of the WNT/beta-catenin pathway.[5,15]

There are notable parallels in the immune phenotype of the MPNST-G1 and -G2 subgroups and previously described classifications. A multi-institutional study of bulk RNA from 64 MPNSTs classified approximately half as "immune active" with favorable prognosis and the other half as aggressive "immune deficient" MPNSTs, with the latter characterized by PRC2 and TP53 loss, expression of proliferative signatures, and high chromosomal instability.[24] Post-hoc analysis of pretreatment biopsies in a negative phase II study of pexidartinib and sirolimus in MPNSTs, where patients had pretreatment and on-treatment tissue biopsies, also noted that immune-depleted MPNSTs had rapid progression and shorter overall survival compared to immune-enriched MPNSTs.[25] Four of the five longest surviving patients in the study had immune-enriched tumors.[25] Overall, these prior bulk studies from human tumor tissue strongly support the existence of two molecular subgroups: a more aggressive immune-deficient MPNST and an immune-infiltrated MPNST with better prognosis.

Single-cell molecular profiling has further characterized these subgroups. While pathway analysis was utilized in prior bulk analysis studies to identify up- and downregulated immune-related pathways and deconvolution algorithms to infer proportion of cell types, scRNA-seq has identified specific cell populations in each subgroup. Expectedly, cell cycle phase estimation on a single-cell level demonstrated that Group 1 MPNSTs are enriched in actively dividing cells.[15] MPNST-G2 and ANNUBP were also comprised of significantly more immune cells compared to MPNST-G1, with a predominance of macrophages followed by T cells.[5] In contrast, MPNST-G1 were largely comprised of proliferating malignant cells and immature Schwann cells, with low immune infiltration.[5,15] Isolated study of the malignant cell populations of MPNSTs revealed a high expression of neural crest cell marker genes among MPNST-G1 tumor cells. Tumor cells of MPNST-G1 were instead observed to have high expression of genes associated with Schwann cell-precursors (SCPs), further differentiated along the neural crest/Schwann cell developmental lineage.[5] Collectively, the integration of bulk and single-cell studies establishes a framework for understanding intertumoral heterogeneity in MPNSTs. MPNST-G1 represents a highly malignant, immune-deficient, primitive neural crest cell-like state and confers a poor prognosis, whereas MPNST-G2 retains features of a more differentiated SCP, an immune-enriched microenvironment, and better prognosis.[24]

Identifying the Cell of Origin

Genetic and lineage tracing studies in genetically engineered mouse models have indicated that MPNSTs and their precursors arise from the neural crest-derived Schwann cell lineage.[17,26,27] In three different murine models of MPNST, transgenic labeling of early neural crest cells identified a common subpopulation of quiescent nestin-positive stem cell-like cells present in MPNSTs that expressed genes characteristic of neural crest and Schwann cell progenitors.[17] Importantly, a transcriptionally similar population was also identified through single-cell profiling of human ANNUBP, the precursor of MPNST.[17] Nestin, a neural crest stem cell marker that has also been described in other malignancies,[28] was therefore highlighted as a marker of the MPNST cell of origin.

Another study performed single-cell characterization of early and late-stage tumors in a murine model of MPNST. In contrast to the previous study, the authors found that nestin expression was a non-specific marker broadly present throughout Schwann cell lineage cell clusters while a distinct nestin-negative mesenchymal neural crest-like (MES-NC-like) population exhibiting a primitive epithelial-to-mesenchymal transition (EMT) signature was exclusive to MPNSTs. They instead described another stem-like population consisting of nestin-negative mesenchymal (MES) neural crest-like cells. This subpopulation exhibited an epithelium-mesenchymal transition (EMT) signature that appeared more primitive than nestin-positive progenitor cells. An analogous population of mesenchymal stem-like subpopulation was present in human MPNST samples and absent in human neurofibroma samples. The MES-NC-like cells were distinct from nestin-expressing cells, instead expressing high levels of ZEB1, an EMT transcription factor.[19] Consistent with these findings, spatial transcriptomics of three MPNSTs later demonstrated regional segregation of tumor subpopulations expressing Schwann cell precursors/neural crest cell-associated genes and those enriched for mesenchymal-neural crest-like gene signatures.[29] Together, these studies suggest that ZEB1 and EMT-associated transcriptional programs may serve as indicators of malignant transformation from plexiform neurofibroma to MPNST. Future investigations correlating the expression of these markers with histologic progression could help establish molecular biomarkers for early detection and risk stratification in patients with neurofibromas at risk of malignant transformation.

Malignant Dedifferentiation Along the Schwann Cell Lineage Trajectory

These observations of dedifferentiated markers amongst MPNSTs have motivated efforts to model the trajectory of dedifferentiation in a variety of ways. Trajectory, or pseudotime analysis, orders cells along a continuous axis based on transcriptional similarity, allowing inference of potential developmental or differentiation pathways. Applied to MPNSTs, these analyses can model how tumor types relate along a continuum of malignant transformation. The two molecular subgroups of MPNSTs reviewed above had expression of markers from different stages of the SC lineage.[5,15] This suggests a progressive model of dedifferentiation, with MPNST-G1 representing a highly malignant, dedifferentiated state, while MPNST-G2 retains features of a more differentiated Schwann cell identity. In concordance with this, trajectory analysis positioned ANNUBP, MPNST-G2, and MPNST-G1 along a pseudotime axis, reinforcing their positions in sequential stages of the neural crest/Schwann cell lineage (Figure 1).[5]

Zhang and coauthors took another approach to trajectory analysis. They leveraged independent publicly available single-cell transcriptomic datasets of neural crest cells, adrenal medulla cells, and mature Schwann cells to model the

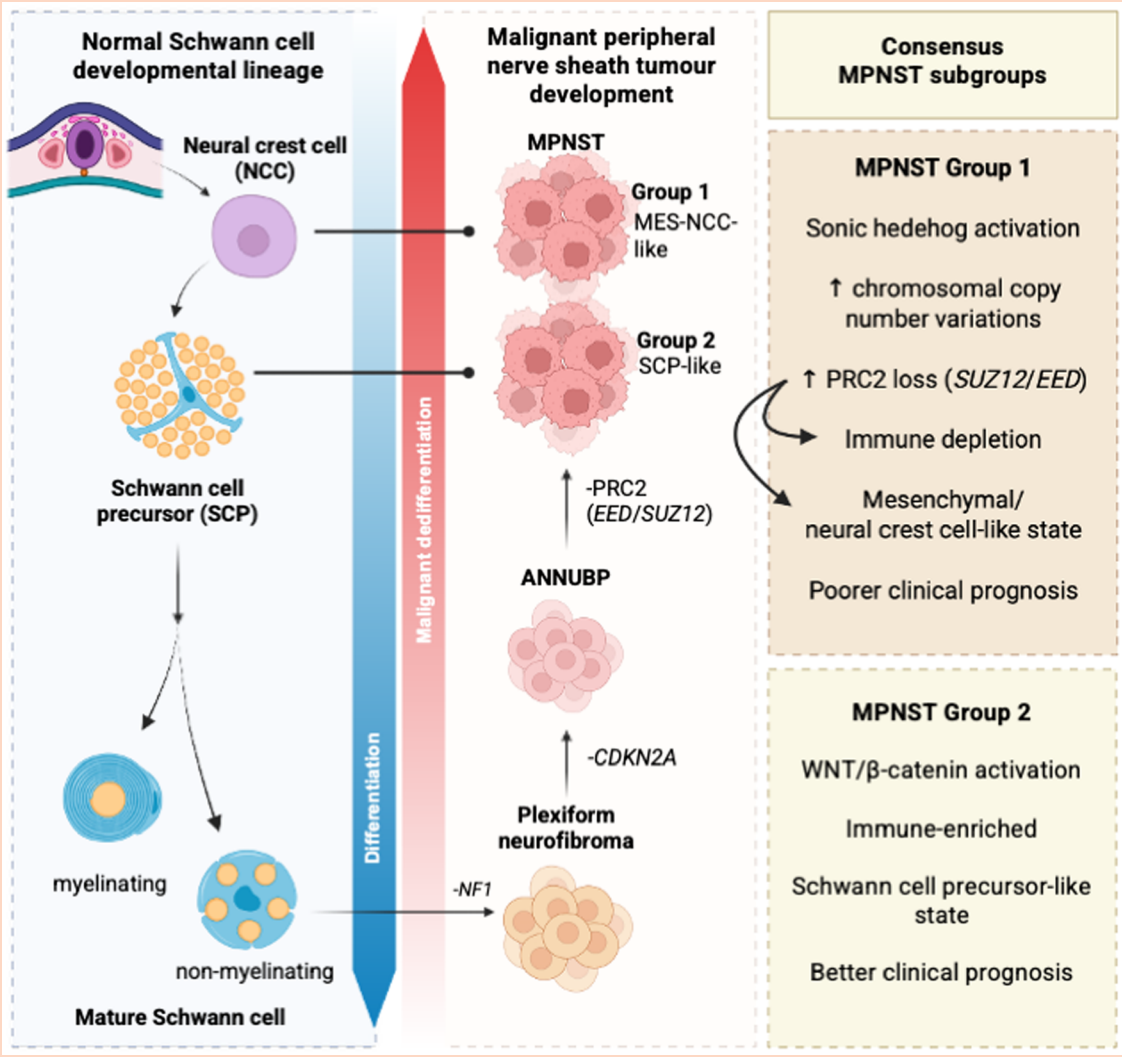

Figure 1. Summary of Malignant Peripheral Nerve Sheath Tumor Development and Subgroup Consensus. Schematic depicting the relationship between normal Schwann cell developmental lineage and stages of malignant peripheral nerve sheath tumor development. During tumorigenesis, progressive malignant dedifferentiation occurs along the neural crest/Schwann cell lineage, with stepwise loss of NF1, CDKN2A, and PRC2 components (*EED* or *SUZ12*) marking key steps from plexiform neurofibroma to atypical neurofibromatous neoplasm of uncertain biological potential (ANNUBP) and MPNST. Group 1 MPNSTs are characterized by PRC2 loss, Sonic hedgehog pathway activation, immune depletion, mesenchymal/neural crest-like programs, and poorer clinical outcomes. Group 2 (SCP-like) MPNSTs show WNT/β-catenin activation, immune enrichment, Schwann cell precursor-like features, and more favorable prognosis. Created in BioRender. Singh, O. (2025) https://BioRender.com/lw26570

normal developmental trajectory of neural crest to Schwann cells.[30] Using this inferred pseudotime model, they identified key genes and transcriptional programs defining sequential steps in Schwann cell maturation. These stage-specific gene set were then examined in normal and malignant cells from scRNA-seq of human MPNSTs. Malignant cells exhibited comparatively higher expression of genes characteristic of non-myelinated SC and mesenchymal neural crest stem cells but lower expression of genes associated with mature myelinated Schwann cells.[30] This approach highlighted the lack of a scRNA-seq atlas spanning the full neural crest-Schwann cell lineage at the time, which necessitated bioinformatic integration of different studies. More recently, an extensive murine atlas encompassing Schwann cell precursors and their terminal fates has been generated, providing a valuable reference for future studies of lineage relationships and malignant transformation in peripheral nerve sheath tumors.[31,32]

Epigenetic Dysregulation

Epigenetic dysregulation is a defining feature of MPNSTs, most notably through loss of PRC2. Bulk transcriptomic analyses comparing PRC2-wildtype or PRC2-deficient MPNSTs have shown that PRC2 loss is associated with broad suppression of immune-related pathways. Genes involved in antigen presentation and interferon signaling were markedly downregulated in PRC2-loss MPNSTs, which also displayed reduced immune cell infiltration.[32] Mechanistically, PRC2 loss induces epigenetic reprogramming that impairs antigen presentation, IFN-γ signaling, and chemokine

production, collectively resulting in an immune-cold tumor microenvironment.[32]

Building on these observations, single-cell sequencing has revealed that PRC2 loss within malignant cells specifically drives activation of cell fate-determining transcription factors through enhancer recruitment while simultaneously suppressing type I interferon signaling.[13,30] Malignant MPNST cells are enriched for PRC2 target genes compared to benign Schwann cells within the same tumors. Expression of genes normally repressed by PRC2 distinctly marks malignant cells, underscoring PRC2 loss as a key event in malignant transformation. Motif analyses of promoter and enhancer regions of MPNST-associated genes using chromatin immunoprecipitation sequencing identified overrepresentation of FOXC1 binding sites. In a separate MPNST sample, FOXC1 was markedly elevated in malignant cells, and the FOXC1 motif was enriched within enhancers of MPNST-specific genes, implicating FOXC1 activation as acts downstream of PRC2 loss in driving malignancy.[30]

Chromatin accessibility profiling further reinforces this model of epigenetic reprogramming of MPNSTs. Using single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq), Wu et al. identified distinct chromatin accessibility landscapes among malignant mesenchymal neural crest-like cells, neoplastic Schwann cells, and non-myelinating Schwann cells in MPNSTs. Neoplastic Schwann cells had open chromatin in genes associated with immature and undifferentiated Schwann cells, whereas mature non-myelinating Schwann cells had significantly more accessible chromatin in glial cell differentiation genes.[19] Collectively, these findings highlight that PRC2 loss and associated enhancer remodeling reshape chromatin landscapes to drive transcriptional reprogramming and malignant transformation in MPNST.

Therapeutic Vulnerabilities and Treatment Response

Treatment resistance remains a significant challenge in the treatment of MPNSTs, and no systemic therapy has yet demonstrated consistent clinical benefit. Selumetinib, a MEK inhibitor, has demonstrated partial efficacy in the treatment of plexiform neurofibromas,[33–35] but preclinical and clinical efficacy in MPNSTs has been limited. Compared to bulk analysis, scRNA-seq can distinguish whether therapeutic pressure acts uniformly across tumor cells or selectively enriches resistant subpopulations. To investigate this, a study analyzed scRNA-seq performed on murine MPNST allografts that were treated with selumetinib or vehicle control. This identified a distinct tumor subpopulation that was enriched in tumors post-treatment, representing selumetinib-resistant cells.[15] The selumetinib-resistant cells were distinct from clusters of differentiated tumor cells and proliferating tumor cells, with decreased expression of proliferation and differentiation genes.[15] Functional studies and CRISPR screening then identified NF2 loss and PAK activation as important mechanisms for selumetinib resistance. Future studies would benefit from mapping the transcriptional signature of the resistant cell population in the murine allografts back to human MPNST scRNA-seq data to identify whether an analogous population may be preexisting in treatment-naïve patients. Furthermore, scRNA-seq data from human tumors of pre- and post-treatment or treatment-resistant patients would be valuable, particularly in the context of the two molecular subgroups that have been described.

The success of immunotherapy in other cancers has also motivated the study of the immune microenvironment and the consideration of immune modulation in MPNSTs. Immunotherapy relies on an immune-enriched microenvironment or a "hot" tumor, and the identification of MPNST-G2 and other similarly described immune-enriched MPNSTs modulation suggest that a subset of MPNSTs may be responsive to immune modulation.[5,24] Zhang et al. performed immunohistochemistry and flow cytometry studies and observed a high proportion of CD163+ and PD-L1-high tumor-associated macrophages and activatedT cells in immune-enriched MPNSTs.[36] In contrast to other studies that suggest immune infiltration is protective,[5,24,25]TAM-infiltration in this study was associated with poorer prognosis.[36] Yan et al. also demonstrated that intratumoral delivery of immunogenic viruses, specifically the inactivated modified vaccinia virus Ankara, may be a strategy to sensitize PRC2-negative tumors to immunotherapy, though this would require validation in an MPNST model.[32]

Future Directions

While single-cell RNA sequencing has significantly advanced our understanding of MPNST heterogeneity and tumor evolution, key questions remain regarding the epigenetic mechanisms that underlie malignant transformation and progression. The rarity of MPNSTs and high quality tissue needed to perform single-cell profiling has been one inherent challenge to this field, as well as the lack of neural crest/Schwann cell developmental atlases until recently.[37]To date, most studies on MPNST scRNA have been small cohorts of less than five samples. While integration of multiple datasets is possible, this may obscure important biological signals, such as the molecular subgroups previously described. Current datasets use a variety of technologies that introduce further batch effect (Table 1). Importantly, spatial transcriptomic studies also require high quality scRNA-seq atlases to use as references, as the field progresses, larger, high-quality atlases may be beneficial.

Given the central role of epigenetic dysregulation and PRC2 loss in MPNST biology, future studies incorporating single-cell methylation and ATAC-seq approaches may be useful to characterize these regulatory landscapes with greater resolution. Single-cell DNA methylation profiling could also uncover lineage-specific methylation patterns, identify early epigenetic alterations that precede malignant transformation, and reveal subclonal epigenetic heterogeneity not detectable at bulk resolution. Similarly, single-cell ATAC-seq would enable mapping of chromatin accessibility in individual cells, helping to define active regulatory elements and transcription factor networks that drive malignancy, particularly in PRC2-deficient contexts.

Integrative multi-omic single-cell approaches combining transcriptomic, epigenomic, and proteomic data will be critical to fully characterize the cellular hierarchies and regulatory programs in MPNSTs. Applying these technologies to primary human MPNSTs, precursor lesions including benign

Table 1. Single-Cell RNA/ATAC Sequencing Studies on MPNSTs and Plexiform Neurofibromas

Study	Samples (species)	Technology	Findings
[5]Suganth et al. Nature Communications 2023	(H) 4 MPNSTs (H) 1 ANNUBPs	snRNA sequencing, 10× Genomics	Group 1 MPNST: immune-depleted, increased NCC marker expression Group 2 MPNST: immune-enriched, better prognosis, increased SCP marker expression
[15]Vasudevan et al. Nature Communications 2024	(H) 3 MPNSTs (Group 1) (H) 3 neurofibromas (M) Murine allograft model pre- and post-MEK inhibitor treatment	snRNA sequencing, 10× Genomics scRNA sequencing, 10× Genomics	Group 1 MPNST: ↑ proliferating tumor cells, immature Schwann cells, growth factor stimulated tumor cells Neurofibromas: ↑ non-tumor cells, non-proliferating cells, non-myelinating Schwann cells Distinct cell cluster enriched in post-treatment tumor
[17]Sun et al. Cell Stem Cell 2021	(H) 1 ANNUBPs	scRNA sequencing, 10× Genomics	Validated the presence of nestin+ cancer stem-like cells identified in murine studies of MPNST as potential cell of origin
[19]Wu et al. Science Advances 2022	(H) 4 MPNSTs, 10 pNFs	scRNA sequencing, 10× Genomics scATAC sequencing, 10× Genomics	MPNST: ↑ ZEB1^{high}, nestin-negative, mesenchymal NCC-like, malignant mesenchymal-like, hypoxic malignant SCP-like states, SPP1^{high} macrophages, ↓ resident fibroblasts pNF: immature SC, mature SC, inflammatory SC with antigen-presenting signature, proinflammatory macrophage, resident fibroblasts Differentially accessible chromatin regions in genes related to embryonic neural crest development, EMT, and mesenchymal pathways in MPNST tumor cells
[23]Mitchell et al. Clinical Cancer Research 2024	Reanalysis of Wu et al. scRNA samples		Proangiogenic markers are highly expressed in MPNSTs, including in tumor cells, macrophage and DC, and FBs
[30]Zhang et al. Cell Reports 2022	(H) 5 paired samples from 2 MPNSTs (biopsy, resection, lung metastasis) (M) SC lineage & trajectory constructed from 3 external references	scRNA of fresh tissue (10× Genomics) single-cell whole genome sequencing (10× Genomics)	MPNST malignant cells: ↑ EMT, embryonic stem cell pathways, FOXC1 targets ↓ immune presentation in metastasis vs. primary malignant cells MPNST cells: ↑ non-myelinated SC, mesenchyme NCC, migratory cell markers (markers from constructed trajectory of mouse SC lineage from external references)
[29]Bremer et al. Neuro-Oncology 2025	(H) 2 hybrid PNSTs, 1 pNF, 1 NF2-associated nerve tumorlets, 3 MPNSTs, 1 primary nerve synovial sarcoma	Spatial transcriptomics, 10× Genomics Visium on FFPE Used Wu et al. scRNA data as reference	SCP/NCC-high and MES-NCC segregate spatially MES-NCC are associated with immune infiltration
[18]Amani et al. Acta Neuropathologica Communications 2023.	8 fresh frozen pNFs	snRNA of 8 fresh frozen pNFs Spatial transcriptomics (10× Genomics Visium Spatial v1 3′ gene expression) on 4 frozen pNFs	Spot analysis: 8-12 cells per 55 um spot spaced 100 um apart Jaccard analysis to compare ST cluster markers gene lists to snRNA seq cellular subpopulation gene markers Spatial intermixing of cellular subpopulations

Abbreviations: ANNUBP, atypical neurofibromatous neoplasm of uncertain biological potential; ATAC, Assay for Transposase-Accessible Chromatin; SCP, Schwann cell precursor; MPNST, malignant peripheral nerve sheath tumor; NCC, neural crest cell; NF2, Neurofibromatosis Type 2; pNF, plexiform neurofibroma; PNST, peripheral nerve sheath tumor; scRNA, single-cell RNA; snRNA, single-nuclei RNA.

neurofibromas and transitional ANNUBPs, and preclinical models may help identify therapeutic vulnerabilities and improve molecular classification. There have been numerous studies using bulk methylation and transcriptomics supporting the existence of two clinically-relevant molecular subgroups of MPNSTs, one that is immune-enriched with a favorable prognosis and another that is immune-depleted with more aggressive behaviour.[5,15] Investigating this further with single-cell data, including methods that examine cell-cell interactions with the tumor microenvironment, would be beneficial in understanding the underlying biology and identifying therapies tailored to distinct subtypes.

Spatial transcriptomic studies in MPNSTs are currently limited but are poised for rapid expansion. Existing work has relied on earlier platforms with relatively large capture spots (55 μm in diameter), where each spot contains transcripts from multiple cells, limiting single-cell resolution and precise cell-type localization. With advances such as Visium HD and Visium HD 3′ technologies, gene expression can now be mapped at or near single-cell resolution while preserving tissue architecture. Recent spatial analyses of plexiform neurofibromas have revealed extensive spatial intermixing of neoplasticSchwann cells and fibroblasts, underscoring the structural and cellular complexity of peripheral nerve sheath tumors.[18] Pilot spatial transcriptomics of MPNSTs have demonstrated segregation of tumor subpopulations with Schwann cell precursors/neural crest cell and those with mesenchymal-neural crest-like gene signatures.[29]This work used the probe-based Visium HD platform, which is optimized for FFPE tissue but is probe-based and therefore to a fixed gene panel. The newer Visium HD 3′ sequencing technology, which captures the entire transcriptome, is designed for discovery applications, but currently only available for fresh frozen tissue, which can be challenging to access. Expanding such spatial transcriptomic work across molecular subgroups and along the trajectory of progression and metastasis will provide critical insight into the spatial dynamics of MPNST evolution.

Finally, translation of these findings to patients with MPNSTs and development of novel therapeutic strategies is needed. Early detection of transformation from plexiform neurofibroma to MPNST remains challenging, with no clear biomarkers or definite imaging characteristics. Liquid biopsy techniques, such as methylation profiling of circulating cell-free DNA released from the tumor into the bloodstream, may be a possible strategy to detect early transformation and identify the molecular subgroup and behavior of the tumor.[38,39]

Conclusion

Single-cell profiling has significantly enhanced our understanding of the molecular and cellular heterogeneity of MPNSTs. These aggressive sarcomas exhibit marked intratumoral and intertumoral diversity, with distinct molecular subgroups that reflect different stages of Schwann cell dedifferentiation. Single-cell analyses have elucidated valuable insights into cellular heterogeneity of MPNSTs and their molecular subgroups, supporting the hypothesis that MPNST pathogenesis parallels dedifferentiation along the neural crest/Schwann cell lineage. Integration of transcriptomic and epigenomic data has further established loss of PRC2 as a central driver of MPNST biology, promoting oncogenic transcriptional programs, suppressing interferon signaling, and shaping an immune-cold tumor microenvironment. Emerging spatial transcriptomic and multi-omic single-cell technologies now offer the opportunity to contextualize these findings within the tissue architecture, enabling integrative analysis of malignant, stromal, and immune compartments at unprecedented resolution. Together, these advances establish a framework for understanding MPNST pathogenesis at single-cell resolution and lay the groundwork for translational progress. Future studies that integrate transcriptomic, epigenetic, and spatial datasets across larger, well-annotated cohorts spanning benign neurofibromas, ANNUBPs, and MPNSTs will be critical to uncover the molecular determinants of malignant transformation, identify biomarkers for early detection, and define actionable therapeutic targets tailored to distinct molecular subgroups.

Keywords

malignant peripheral nerve sheath tumor | MPNST | neurofibroma | neurofibromatosis | single-cell

Conflict of Interest Statement

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