BACKGROUND: Medulloblastoma defines a heterogeneous set of neuro-epithelial neoplasms of the posterior fossa. Four groups of medulloblastoma are recognized: wingless (WNT), sonic hedgehog (SHH), group 3, and group 4. The tumor microenvironment (TME) influences tumor progression and response to therapy and has emerged as a growing target for the study of novel therapeutic approaches. METHODS: We show spatial gene expression architecture through 10X Visium Spatial Sequencing on 16 formalin-fixed paraffin-embedded (FFPE) samples representing all molecular groups of medulloblastoma. Spatial transcriptomics data is correlated with clinical parameters, including M-stage at diagnosis, tumor histological classification, and outcomes data including survival and relapse status. RESULTS: Spatial sequencing demonstrates both intra- and inter-tumoral TME heterogeneity across molecular subgroups. Unsupervised clustering and uniform manifold approximate projection illustrate clusters of distinct spatial gene expression representing cell states from different components of the TME, including tumor-associated astrocytes (TAA), macrophages (TAM), and vascular endothelium. Medulloblastoma cells constitute the majority of cells and express marker genes representing different stages of neuronal differentiation and cell cycle. Pathway analysis reveals the enrichment of pathways associated with cell mortality (Rho GTPase, MET, MAPK signaling), heat shock response, growth factor signaling, and adaptive immune response (TLR, MHC-II signaling). In addition, molecular pathways known in medulloblastoma, including NF-kB and TP53 regulatory pathways, were also enriched. Neighborhood-enrichment analysis reveals the co-localization of TAMs and TAAs in close spatial relationship with mitotic progenitor-like medulloblastoma cells. Additionally, these two cell types constitute a greater proportion of the TME in patients at relapse compared to initial diagnosis. CONCLUSION: Spatial transcriptomics enables new insight into the heterogeneity of the medulloblastoma TME. Notably, our analysis elucidates the spatial association of TAMs and TAAs with proliferating tumor cells and an increased abundance of these cell types following relapse. These initial results support the role of TAMs and TAAs in medulloblastoma progression.