Bioinformatic Insights into DC3 Dynamics in Advanced Non-Small Cell Lung Cancer: Integrating Single-Cell RNA Sequencing and Spatial Technologies

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Abstract:

Conventional dendritic cells (cDCs) are vital for initiating adaptive immune responses, comprising cDC1s and cDC2s, the latter further divided into DC2s and DC3s. DC3s have emerged as noteworthy contributors to chronic conditions such as lupus, psoriasis, and severe cases of COVID-19. Their implication in these diseases underscores the crucial role of DC3s in immune dysregulation and inflammatory responses. Understanding the specific involvement of DC3s in these contexts offers potential insights for targeted therapeutic interventions and advancements in managing chronic disorders.

Given the chronic nature of lung cancer, our investigation delved into the potential involvement of DC3s, utilizing publicly available single-cell RNA sequencing datasets of non-small cell lung cancer (NSCLC). Our analysis unveiled an enrichment of DC3s displaying a potential immunosuppressive phenotype, especially in advanced disease stages, accompanied by a reduction in the frequencies of DC2 and cDC1 subsets. Employing cross-species single-cell RNA sequencing on human and mouse lung cells, we identified murine DC3s and established a flow cytometry-based method for distinguishing cDC1s, DC2s, and DC3s. This approach was validated by confirming DC3 enrichment in an immune checkpoint blockade (ICB)-resistant adenocarcinoma mouse model, mirroring trends observed in advanced human lung adenocarcinoma.

To gain a thorough insight into the spatial dynamics of DC3s and their molecular characteristics in lung cancer, we incorporated cutting-edge technologies, including the "Padlock" and "CosMx" methods. The Padlock technology, an in situ sequencing technique, allowed for the simultaneous and highly specific detection of multiple DNA or RNA sequences. This capability enabled us to precisely identify the molecular signatures specifically associated with DC3s in the intricate landscape of lung cancer. Concurrently, the integration of CosMx technology facilitated the simultaneous visualization of various biomolecules within tissue samples, providing a spatially resolved map of gene expression.

By combining the strengths of Padlock performed on mouse samples and CosMx technologies applied to human tumor tissues, our approach aims to bridge the gap between detailed molecular information and the spatial context. This integration enhances our capacity to unravel the complex dynamics of DC3s within the tumor microenvironment. Collectively, these advanced techniques propel our research toward an enhanced comprehension of the cellular and molecular intricacies that govern the immune response in the context of lung cancer. Understanding the spatial environment of DC3s is crucial in elucidating their potential interactions and functional roles within the broader context of the tumor microenvironment.