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INVESTIGATING FIBROBLASTIC RETICULAR CELLS (FRCS) REMODELING WITHIN MELANOMA TUMOR-DRAINING LYMPH NODES

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Background. Immune checkpoint inhibitors have significantly improved survival for melanoma patients, however 40-60% remain unresponsive to treatment due to resistance mechanisms[1]. Identifying these mechanisms and developing novel therapies is crucial for enhancing therapeutic benefits.

Tumor-draining lymph nodes (TDLNs) act as an extension of the tumor microenvironment, facilitating tumor dissemination and influencing local and systemic immunity[2]. A comprehensive understanding of TDLN remodeling in melanoma is essential for elucidating the immunological mechanisms driving the formation of metastatic niches and tumor progression, and response or resistance to current immunotherapies. Immune-specialized fibroblastic reticular cells (FRCs) within LNs play a critical role in organizing immune cells and regulating local and systemic immunity[3], potentially orchestrating anti-tumor immune responses in melanoma

Material and Methods. To investigate the status of TDLN-FRCs in human melanoma, we conducted multiplexed immunofluorescence coupled with bioinformatic analysis on formalin-fixed paraffin embedded (FFPE) lymph node tissues from healthy controls (n=6), and melanoma patients (n=19). Tissues were stained with PDPN, CD31, and aSMA antibodies to identify FRCs ($PDPN^+CD31^-$), excluding lymphatic and blood endothelial cells (LECs: $PDPN^+CD31^+$, BECs: $PDPN^-CD31^+$). In addition, to further characterize melanoma TDLN-FRCs, we performed ex vivo morphology and transcriptomic analysis of

primary human FRCs freshly isolated from stage IIIC melanoma patients undergoing lymphadenectomy, comparing them to FRCs from healthy individuals.

Results. Computational analysis of FFPE LN tissues revealed a remodeled FRC network co-expressing aSMA$^+$ in melanoma TDLNs, with larger spaces between reticular branches, suggesting loosened FRC network similar to that observed during immune responses or in pathological conditions[4-6].

To assess melanoma-induced FRC activation, we co-cultured primary human FRCs with melanoma cell lines A375 and SK-MEL-24 (to mimic a metastatic LN) or conditioned them with melanoma supernatant (to mimic a non-metastatic LN). Morphology analysis of healthy human FRCs conditioned with melanoma cell lines, revealed that both experimental conditions induced FRC relaxation.

To confirm if similar activation occurred in patient TDLNs, we isolated FRCs from stage IIIC melanoma patients undergoing lymphadenectomy. Cell shape assessment revealed a consistent stretching of melanoma FRCs compared to healthy counterparts.

To gain deeper insights into melanoma-induced remodeling of TDLN FRCs, we performed RNA sequencing on freshly isolated FRCs from melanoma TDLNs (5 donors with stage III melanomas) and compared their transcriptional profiles to healthy FRCs. Our analysis revealed enrichment in cell proliferation, metabolism, histone modification, and extracellular matrix remodeling pathways, alongside with downregulation of genes associated with cholesterol metabolism and collagen secretion. Additionally, we observed changes in immunomodulatory genes linked to MHC II (upregulation) and chemokines (CCL2 and CXCL12 downregulation).

Conclusions. These findings suggest that melanoma alters TDLN-FRCs potentially through soluble factors promoting network remodeling and transcriptional reprogramming. Further research is undergoing to elucidate the impact of melanoma-FRCs remodeling on TDLN immunity and, potentially, on tumor progression and response to immunotherapy.

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