Spatial mapping of macrophage heterogeneity in murine atherosclerotic plaques

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Atherosclerosis-related CVDs are the leading causes of death worldwide. As the most abundant immune cell type in plaques, macrophages play crucial roles in the atherosclerotic pathogenesis. Due to their versatile and plastic phenotype in response to locally-produced factors, they are very heterogeneous and can exert both pro- and anti-atherogenic activities. As context, and hence macrophage heterogeneity, is highly disease stage-dependent, this considerably complicates the design of effective macrophage-targeting therapies. To fully dissect their impact and the different subsets' individual roles in the pathogenesis, a complete and comprehensive mapping of the intraplaque myeloid phenotypic landscape would be necessary.
In recent publications, newly-emerging approaches such as mass cytometry and single-cell RNAseq underpinned this heterogeneity, but interpreting the classification's functional implications remains difficult since both techniques suffer from the same caveats: not only is all spatial information on the cells' location lost during tissue disruption steps to obtain single-cell suspensions, specific subsets (e.g. the fragile foam cells) will also be lost during this procedure, leading to an underestimation of their contribution to the disease progress. In immunohistochemistry (1-2 colors) or immunofluorescence (3-5 colors) on the other hand, no cells are lost and all information on their location is retained, but these methods lack the high phenotypic resolution required to distinguish the different subsets.
We therefore developed a 15-color multispectral imaging approach on murine plaque sections, as well as the cell clustering and micro-environmental mapping tools for subsequent analysis, and applied these on 148 plaque images identifying over 88,000 individual cells. This resulted in the characterization of plaque myeloid heterogeneity with a phenotypical resolution comparable to cytometry but avoiding the cell detection bias resulting from tissue processing, and retaining all spatial information on each individual cell. This way we can quantify the phenotypes' co-localization with other cell types (cell communities) or with plaque domains (niches) and characterize the (molecular and/or metabolic) micro-environment in adjacent tissue sections, shedding light on their influence on the plaque macrophages' phenotypes and functions.

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