Towards the complete picture: computational strategies to identify MS-Imaging derived molecular fingerprints associated with inflammatory cell components

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Background and Objective

Macrophages can adapt to stimuli in their direct environment, adopting a functional phenotypic spectrum throughout the disease course of atherosclerosis. While this suggests significant plaque macrophage heterogeneity, this has thus far only been poorly described, largely due to technical constraints. Here we present a new strategy to dissect macrophage heterogeneity in its molecular context at high spatial resolution.

Methods

Fig. 1: (a) Data collection. (b) Pipeline.

Results

Clustering analysis

Overlaying a cell segmentation mask on 12 phenotype-defining fluorescent channels resulted in a matrix of mean fluorescent intensities of these markers for each individual cell. Cell segments were grouped into 30 clusters, using a cosine similarity-based K-medoids approach.

Fig. 2: Visualization of clustering results shown on tSNE (a), minimal spanning tree (b), k-nearest neighbor network ((c), k=3), histogram on each channel (d), violin plot (e). Each cluster can also be overlaid on the H&E image for pathological analysis (f).

Image registration

Fig. 3: Two sections were aligned by a series of (rigid and elastic) image registration algorithms, mapping the positions of the identified macrophage clusters on the MSI dataset to label each MS pixel a certain population.

Feature selection

Fig. 4: (a) Several of the 30 cell clusters were combined based on domain knowledge and hierarchical clustering (b), to select the most significant features associating with these combined clusters. (c) & (d): M/Z and tSNE of the top 6 features we selected.

Conclusion

We have developed an innovative new approach to define not only cell heterogeneity, but also to link this to the microenvironment. The methodology is also applicable to other cell types and tissue and may lead to a breakthrough in linking molecular context to cellular phenotype and function, in healthy and diseased tissues.

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