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# Characterization of cancer-associated adipocytes by Raman spectroscopy

Adipocytes are the major cellular components comprising the breast cancer microenvironment. In the early stage of breast cancer, cancer cells locally infiltrate the nearby adipose tissue, which results in the activation of adjacent adipocytes into cancer-associated adipocytes[1] (CAAs). Consequently, it gives advantages to breast cancer cells in terms of survival, growth, and metastasis. Compared to normal adipocytes, CAAs are mainly characterized by a decrease in size, lipid content, adipocyte differentiation markers, and an increase in adipokines and inflammatory factors. We propose to use Raman spectroscopy to identify new biomarkers representative of the status of CAAs.

To capture transition between adipocyte and CAAs, a 2D in vitro co-culture model, providing a contact area between adipocytes (differentiated 3T3-L1 cells) and breast cancer cells (MDA-MB-231, Claudin-low subtype) was developed. However, cell heterogeneity is a major drawback of this cell culture model. For example, each kinetic time is marked by the coexistence of immature (fibroblast-like) and mature (adipocyte-like) 3T3-L1 cells non-equally differentiated. On the other hand, the adipocyte/CAAs transition induced by breast tumor cells varies from one adipocyte to another. To address this issue, we adapted algorithmic tools, recently implemented for single cell transcriptomic analysis, to vibrational Raman spectral data. These tools, known as trajectory inference (TI) or pseudotemporal ordering[2], aim to reconstruct evolving pathways from different cell states, coexisting simultaneously in a cell population. Our research focused on the use of Partition-based graph abstraction[3] (PAGA) algorithm combined to uniform manifold approximation and projection (UMAP) to decipher adipocyte cells heterogeneity and highlight CAAs population.

We show how Raman spectroscopy can be used in association with TI approach to visualize and resolve the cell heterogeneity of this cell model. More specifically, the results support the evidence of diverse differentiated adipocyte subtypes and CAAs subpopulation. This new approach will pave the way for a better comprehension of cell heterogeneity and may reveal new molecular states and subpopulation-specific responses to external perturbations.

## References

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