Heterotypic spheroids as an advanced cellular model to study metabolic rearrangements in bladder cancer progression

Giulia Gigliotti[1,2], Francesco Cavallotto[1], Giacomo Ducci[1,2], Gloria Campioni[1,2], Valentina Pasquale[1,2], Simone Ponzetto[1,2], Alessia Ruggiero[1], Marco Barreca[1], Daniele M. Papetti[3], Chiara Damiani[1,2], Rocco Piazza[4], Daniela Besozzi[3], Marco Vanoni[1,2], Elena Sacco[1,2]

Email: giulia.gigliotti@unimib.it

[1] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan
[2] SYSBIO-ISBE-IT-Candidate National Node of Italy for ISBE, Research Infrastructure for Systems Biology Europe, Milan
[3]Department of Informatics, Systems, and Communication, University of Milano-Bicocca, Milan
[4]Department of Medicine and Surgery, University of Milano-Bicocca, Monza

Keywords: bladder cancer, heterotypic cancer model, advanced multi-cellular in vitro models, co-cultures, metabolic rewiring

Abstract:
Tumor heterogeneity is one of the most challenging features of cancer and the primary cause of the failure of conventional therapies[1]. Therefore, it is essential to rely on preclinical models better mimicking the tumor mass *in vivo*, characterized by three-dimensional architecture, nutritional gradients, and complex, direct and indirect, interactions between cancer cells and other cell types present in the tumor microenvironment[2]. Metabolic reprogramming is one of the most studied hallmarks of cancer as it is useful for the identification of new potential prognostic markers and therapeutic targets [3].
This work aims to study how the presence of a stromal component can influence tumor physiological and metabolic alterations using heterotypic spheroids from a panel of six bladder cancer cell lines at different stages and grades, together with human primary bladder fibroblasts. To face the complexity of metabolism a systems metabolomic approach integrating omic and morpho-functional data using mathematical modeling and computational simulations will be used. Morpho-functional data (proliferation, apoptosis, invasion, redox homeostasis), possibly with spatial resolution, will be obtained using quantitative imaging techniques based on confocal and II photon microscopy. Spatial transcriptomics will be used to uncover the transcriptional rearrangements occurring in spheroid subpopulations, which will be integrated into a mathematical model of metabolism to predict metabolic rearrangements. These predictions will be functionally validated using Seahorse and IBEX technology. In conclusion, our work will contribute to understanding the molecular and metabolic mechanisms underlying the mutual influence of different cellular components in tumor mass during bladder cancer progression.

1. Duval, K. *et al.* Modeling Physiological Events in 2D vs. 3D Cell Culture. *Physiology* 32, 266–277 (2017).
2. Shannon, A. E., Boos, C. E. & Hummon, A. B. Co-culturing multicellular tumor models: Modeling the tumor microenvironment and analysis techniques. *Proteomics* vol. 21 Preprint at https://doi.org/10.1002/pmic.202000103 (2021).
3. Hanahan, D. & Weinberg, R. A. Hallmarks of Cancer: The Next Generation. *Cell* 144, 646–674 (2011)