PROJECT

Supervisor:	Giovanni Tonon
Title:	Unraveling novel co-dependencies in metastastic colon cancer
Curriculum:	Basic and Applied Immunology and Oncology
Link to the personal page of the University or relevant hospital site website:	https://www.unisr.it/en/docenti/t/giovanni-tonon https://research.hsr.it/en/divisions/experimental-oncology/functional-genomics-of-cancer/giovanni-tonon.html

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Metastatic colorectal cancer (mCRC) remains in most cases uncurable. As in the last decades the therapy has remained largely the same, there is an urgent need to find new, more effective treatments for this cancer. The tumor microenvironment (TME) also shapes the epigenetic and transcriptomic profiles of cancer cells, promoting drug resistance. This study seeks to uncover new vulnerabilities and dissect molecular mechanisms of resistance in mCRC cancer cells.

Rationale and hypothesis

We have accrued hundreds of clinically annotated patient-derived organoids (PDOs), which recapitulate very closely the biology of the underlying tumors. In a subset of them, extensive multi-omic data are available. We have also developed a MicroFluidic Platform (MFP)[1] that allows high-throughput screening of hundreds of PDOs over 384 plates, where drug concentrations could be modulated in each well and eluate retrieved. Retrospective and prospective clinical studies in mCRC confirm that the MFP results closely match the patient responses. The platform supports genetic manipulation of PDOs and deep multi-omics, including whole-genome sequencing, transcriptomics, proteomics, lipidomics, and metabolomics, as well as analyses capturing the epigenetic landscape of cancer cells, leveraging a technique developed in the lab, scGET-seq[2]. We have also implemented in the MPF phenotypic screens leveraging Cell Painting (CP)[3], to perturbations across eight subcellular components, revealing vulnerabilities not captured by proliferation assays. **Leveraging on these unique capabilities, we aim to identify**

new molecular targets and related compounds to dramatically improve the treatment of mCRC.

Objectives and specific aims

By integrating CRISPR/Cas9 screens with clinically centered multi-omic data, and taking advantage of MFP, we seek to unveil in PDOs cancer dependencies beyond those driven by genetic lesions of cancer genes.

Drawing on paradigms of addiction, synthetic lethality and composite markers, in collaboration with Dr. Iorio at Human Technopole[4], we have identified a highly curated list of clinically relevant, druggable targets, linked to biomarkers clinically exploitable. Genes include canonical oncogenic nodes validating the screening platform and novel, druggable targets related to adhesion signaling, cellular plasticity, and stress adaptation that will be tested and validated, using vectors harboring guide RNA sets, alongside drugs identified via DepMap[5] and Connectivity Map[6] integrations. We will follow a stepwise prioritization strategy, progressively focusing on the most informative targets, PDO models, and ecosystem states emerging from each analytical iteration. PDOs grown within MFP will be infected with arrayed CRISPRi and CRISPRa libraries targeting these hits, in collaboration with the Aguzzi lab.[14]

Expected outcomes

We propose a comprehensive strategy aimed to validate novel targets to usher more effective therapies for one of the deadliest cancers.

Skills that the student should acquire (max. 600 characters including spaces):

The student will be exposed to a broad range of genomics approaches, alongside novel molecular and cell biology technologies that allow the comprehensive definition of the (epi)genetic and transcriptomic landscapes. We will exploit engineered in vitro models, patient derived organoids, and a patented microfluidic platform developed in the lab, which allows the concomitant assessment of hundreds of organoids.

References (max. 15)

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