Computational and experimental approaches to discover anticancer targets and therapies

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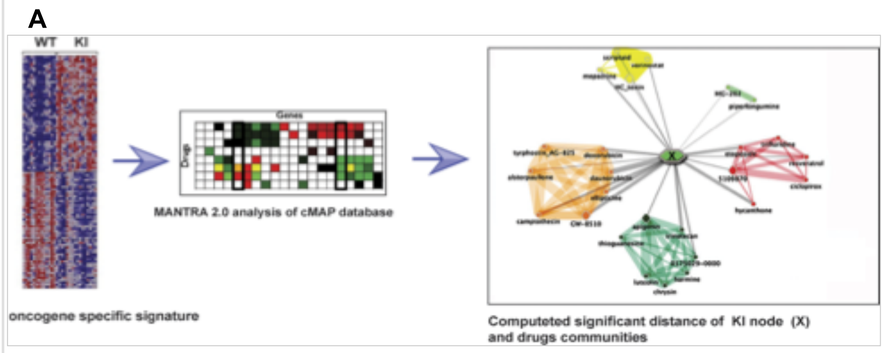
Background. Our laboratory is dedicated to the discovery and targeting of mechanisms relevant to tumor progression and metastatic dissemination. We are currently focused on two malignancies: i) Pancreatic Ductal Adenocarcinoma (PDACs) and ii) metastatic Triple-Negative Breast Cancer (TNBC), that are tumors with limited therapeutic success and poor prognosis. We are also focusing our attention on the potential therapeutic targeting of aggressive cancer stem (CSC) sub-population that is mainly responsible for chemoresistance, tumor relapse, and metastases. Once a target is identified, and if orphan for specific inhibitors, our research aims to identify and repurpose potential novel inhibitors among FDA-approved drugs, with the aim to accelerate the clinical opportunities and the benefit for patients

Project 1 . Effective drug repurposing of KRAS inhibitors

The mutated K-Ras protein represents a pivotal tumor driver in pancreatic, lung and colon cancer. However, despite comprehensive efforts, effective therapeutic inhibitors for mutated K-Ras have yet to reach the clinic. Recently, we have successfully implemented a computational approach based on the investigation of a gene expression signature-based drugs network to in-silico repurpose FDA-approved drugs as inhibitors of mutated K-Ras. We have demonstrated the cytotoxic activity of a specific FDA-approved drug in selected pancreatic cancer cell lines, representing a promising anticancer treatment.



e-PDAC (Kras-dep)	K-RAS mut
HupT4	G12V
Panc08.13	G12D
HPAF II	G12D
PaTu 8902	G12V
Capan1	G12V

m-PDAC (KRAS indep)	K-RAS mut
KP4	G12D
PaTu8988T	G12V
SW1990	G12D

Figure 2. A. Schematic representation of the computational repurposing based on the generation of KRAS-G13D-gene signature (node) and the inspection of a drug-gene network. B. List of PDAC cell lines used in these studies showing differential KRAS dependency according to Singh et al, 2009. C-E. By this approach, we have identified an FDA-approved drug with the potential of inhibiting selectively the growth of PDAC cells, both in vitro (C-D) and in vivo (E).

Perspectives.

By merging computational analysis of "Big data" with pre-clinical validations, our lab aims to address, through cost-effective approaches, the repurposing of FDA-approved drugs for relevant anticancer targets, yet orphans for inhibitors. In the close future, we plan to apply the established research platform to foster the research of immunotherapy unit to understand relevant targets for immune-checkpoint inhibitors therapies resistance in melanoma and lung cancer .

References

1- Carrella D. et al, 2016, Oncotarget.
2- Cardone L. , 2017, Aging.
3- Singh C. et al, 2009, Cancer Cell.
4- Yizhak K. et al, 2013, Nature Communications.

Methods. Our laboratory implies both *dry* and *wet* approaches. We apply mathematical algorithms and bioinformatics approaches based on gene expression data to gather information on oncogenes pathways activation (*Project 1*) or to generate mathematical models of metabolic networks and predicts the activation of druggable metabolic pathways (*Project 2*). Biochemical-, cellular- and mice models-based studies are then applied for the preclinical validation of targets.

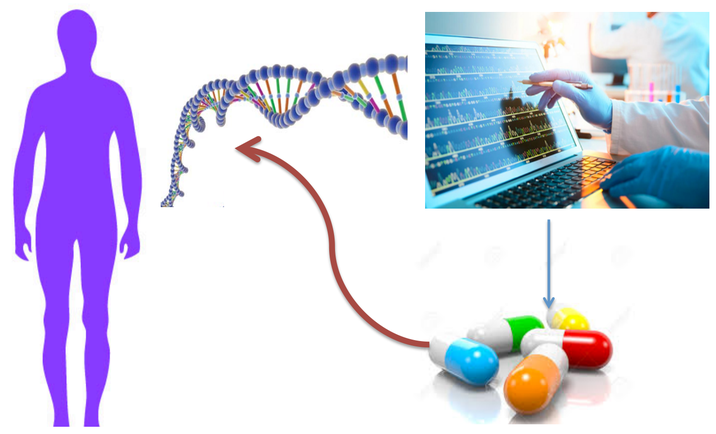


Figure 1. The lab' leading idea: *Big Data* analysis such as transcriptomics, metabolomics and epigenomics can speed-up drug discovery and drug repositioning as well as help tailoring therapeutics for a personalized medicine

Project 2. Identifying and targeting metabolic addiction/s in TNBC-CSCs.

We have recently generated the metabolic network of metastatic CSC derived from TNBC Myc-overexpressing cells, tumors with high metastatic potential and chemoresistance. Our data allowed identifying specific metabolic vulnerabilities, whose targeting limited CSC migratory phenotypes and survival. These pathways thus represent good targets to be exploited in the clinical settings.

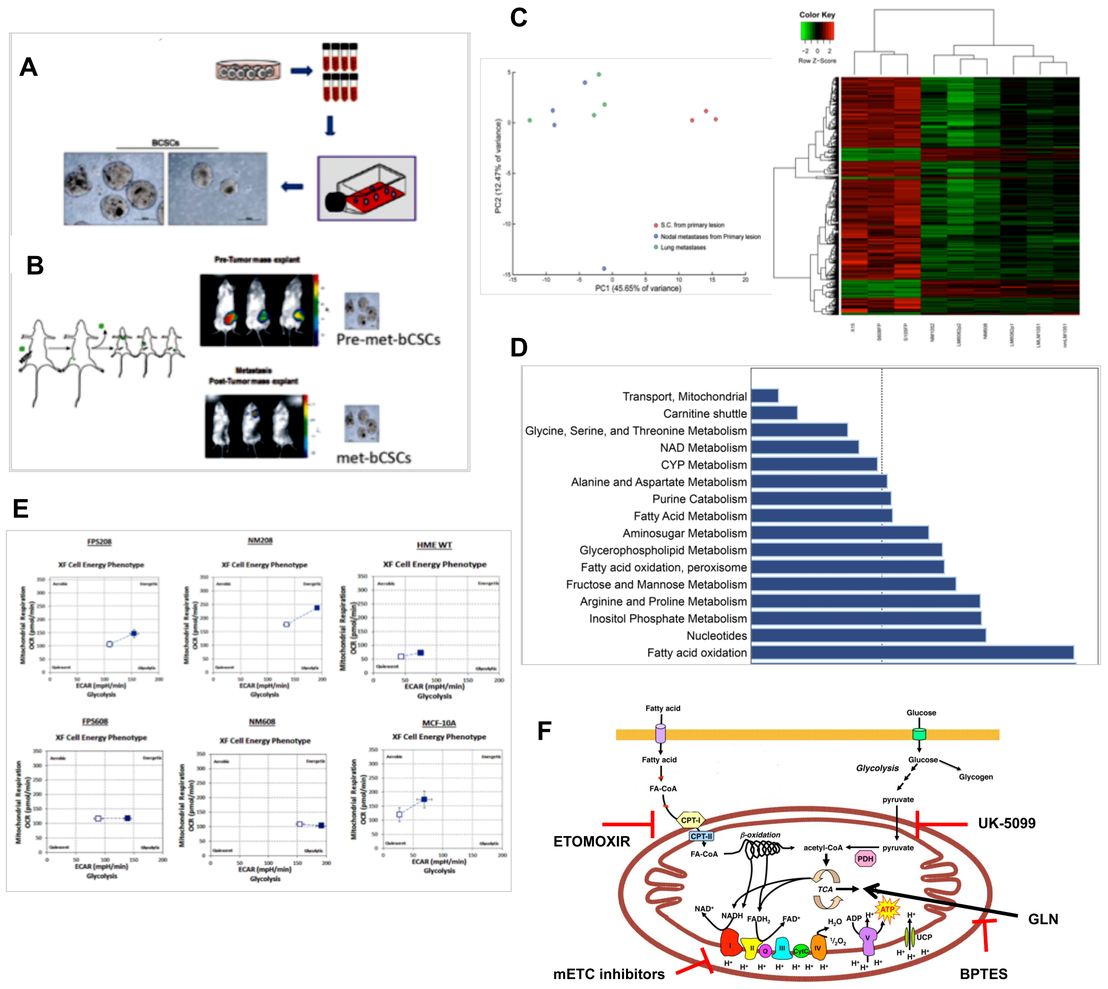


Figure 3. A. bCSCs were isolated from SUM159-TNBC cell lines by mammospheres growth. **B.** bCSCS-enriched mammospheres were then derived from both primary and metastatic tumors. **C-D.** Gene expression profiles, derived from these models, were analyzed by PCA (C) and by cluster analysis (D) before generating a metabolic model (MTA, *Yizhak K et al, 2013*) to predict major metabolic pathways activation (D). **E.** Seahorse XF-flux analyzer confirmed high glycolytic and OxPhox rate in bCSCs mammosheres. **F.** Our analysis provided a rationale to target actionable metabolic pathways by selective inhibitors as potential metabolic vulnerability of CSCs.