TARGETING THE METABOLIC VULNERABILITIES OF METASTATIC COLORECTAL CANCER

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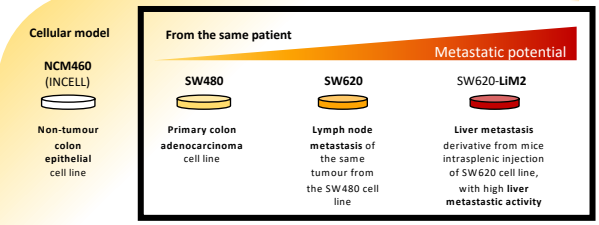
INTRODUCTION AND OBJECTIVES

Metastasis is the main cause of cancer death and its mechanisms are still poorly understood. A better comprehension of the process of how the disseminated tumour cells manage to survive in the circulation and initiate new tumours would help to develop new therapies. Colorectal cancer is the second leading cancer in mortality, with the vast majority of deaths attributable to distant metastasis (Zarour et al., 2017). Genetic alterations seem to be the main driving force for tumourigenesis, however, few genetic changes have been identified during the metastatic process and epigenetic or metabolic changes have emerged as hallmarks of metastasis (Kreuzaler et al., 2019). Prior research in that field has focused more on breast cancer metastasis (Davis et al., 2020; Elia et al., 2019) than on other types of cancer metastases. Colorectal carcinogenesis is commonly driven by the inactivation of the adenomatous polyposis coli (*APC*) tumour suppressor gene and the activating mutation of the oncogene *KRAS*. Metastasis frequently occurs in the close lymph nodes and in the liver through the portal system, and also in the lung, bone or brain. The mechanisms that allow liver and lung colonisation are starting to be elucidated (Urosevic et al., 2014) and we still lack therapies that specifically target cells that have acquired metastasis capacities.

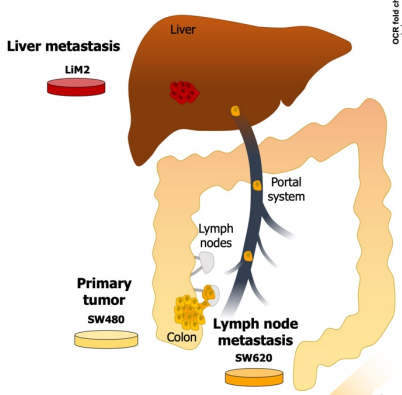
In the present study we hypothesise that a **thorough metabolic characterisation** of the **lymph node** and **liver** derived **metastatic colorectal cancer cell lines** will reveal **specific metabolic fingerprints** that colorectal cancer cells need to acquire in order to generate distant metastases. A multi-omics integration approach using **genome-scale metabolic modelling** (GSMM) (Nilsson et al., 2017) is applied in order to find possible metabolic vulnerabilities of our model that could be **potential targets for metastatic colorectal cancer therapies**.

METHODOLOGY



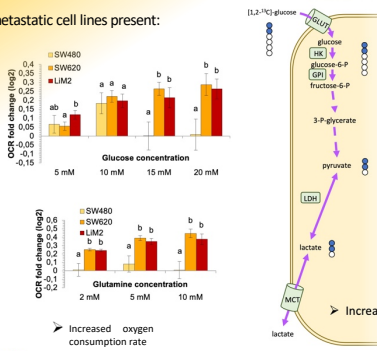
To characterise the metabolic signature of colon cancer metastasis we use the **highly-valuable same patient-derived SW480** (primary), **SW620** (metastatic) together with the metastatic-enriched colon cancer cell subpopulation SW620-**LiM2** (Urosevic et al., 2014).

A full metabolic characterisation is performed including **respirometry assays**, metabolite consumption and production rates, **^{13}C-based metabolomics** using GC-MS, **targeted metabolomics** using UHPLC-MS/MS, together with other molecular biology techniques.

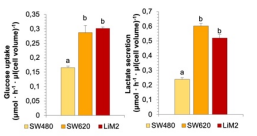


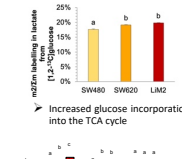
FULL METABOLIC CHARACTERISATION

The metastatic cell lines present:



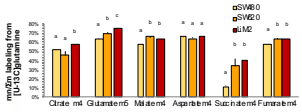
➢ Increased oxygen consumption rate

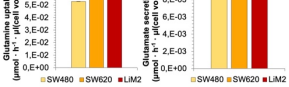


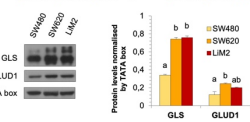


➢ Increased glycolysis

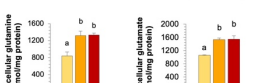
➢ Increased glucose incorporation into the TCA cycle



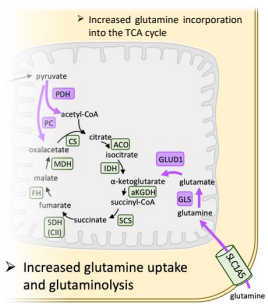




➢ Increased GLS and GLUD1 expression and intracellular levels of glutamine and glutamate



➢ Increased glutamine incorporation into the TCA cycle

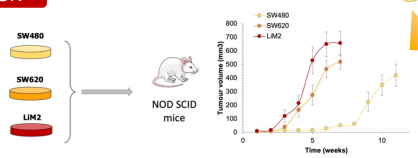


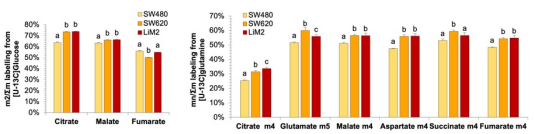
➢ Increased glutamine uptake and glutaminolysis

^{a,b,c}A **one-way ANOVA** was performed for the factor "cell line", and Scheffe's test was used for **multiple comparisons**. Groups sharing the same letter don't show a significant difference with α=0.05.

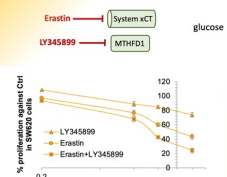
IN VIVO VALIDATION

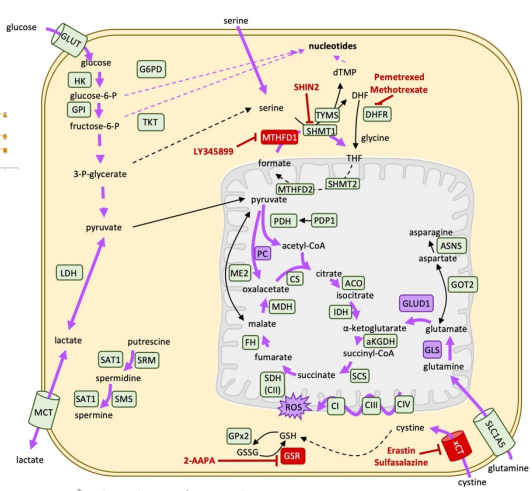
The metabolic phenotype of the metastatic cell lines was confirmed in an *in vivo* model:





TARGET VALIDATION

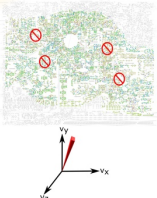




➢ The combination of erastin and LY345899 is synergetic selective for the metastatic cell lines

COMPUTATIONAL MODEL FOR TARGET PREDICTION

The metabolomics and transcriptomics data was integrated in a genome-scale metabolic model:



Knock out simulation

Gene essentiality in a specific cell model

- Synthetic lethal pairs
- Single targets

Model predictions:

Synthetic lethal pairs (gene symbols)	The predicted fraction of growth compared to wild type		
	SW480	SW620	LiM2
SLC7A9, SLC3A2	86%	0%	0%
SLC3A1, SLC3A2	86%	0%	0%
SLC7A5, SLC7A11	85%	0%	0%
SLC7A11, SLC3A2	85%	0%	0%
ETFA, GPI	49%	10%	9%
ETFB, GPI	49%	10%	9%
AGXT, GPI	49%	9%	8%
GPI, GRHPR	49%	9%	8%
CTH, GPI	42%	4%	7%
DLST, GPI	40%	5%	7%
GPI, OGDH	40%	5%	7%
GPI, SUCLG1	35%	1%	1%

Cysteine metabolism

Folate metabolism

Single targets (gene symbol)	The predicted fraction of growth compared to wild type		
	SW480	SW620	LiM2
MTHFD1	100.0%	0.0%	0.0%
GSR	99.0%	0.0%	0.0%
SCD	25.4%	0.0%	0.0%
DLD	5.5%	0.0%	3.5%
PKM	2.3%	0.0%	0.0%
PRODH	0.0%	0.0%	0.0%
GAPDH	0.0%	0.0%	0.0%
GPT	0.0%	0.0%	0.0%
RRM1	0.0%	0.0%	0.0%
GUK1	0.0%	0.0%	0.0%

CONCLUSIONS

- Cells from metastatic sites SW620 and LiM2 have a metabolic reprogramming characterised by **enhanced Warburg effect, glutaminolysis, TCA and mitochondrial activity**.
- The metastatic and metabolic characteristics observed in the cell lines *in vitro* are **maintained** in an ***in vivo*** scenario, which gives more clinical value to such findings.
- A **systems biology approach** based on the integration of metabolic and transcriptomic data in cell line-specific GSMM was a **successful strategy to predict putative drug targets**.
- Inhibitors of the predicted targets in the **cysteine transport and folate metabolism** have been experimentally validated and the combination of specific inhibitors against system xCT and MTHFD1 is **synergetic and specifically affects the metastatic colon cancer cell lines**.

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