Metabolic Rewiring in Metastatic Melanoma Progression

New Prognostic and Therapeutic Targets

UNIPhD
UNIVERSITY OF PADUA PHD PROGRAMME

INTRODUCTION

- Cutaneous melanoma is a highly **aggressive** type of skin cancer triggered by **UV** exposure.
- Metastatic spread, characterised by the transition of primary tumour cells into circulating tumour cells and then secondary tumours, is associated with **severe morbidity and mortality** with **limited treatment options.**

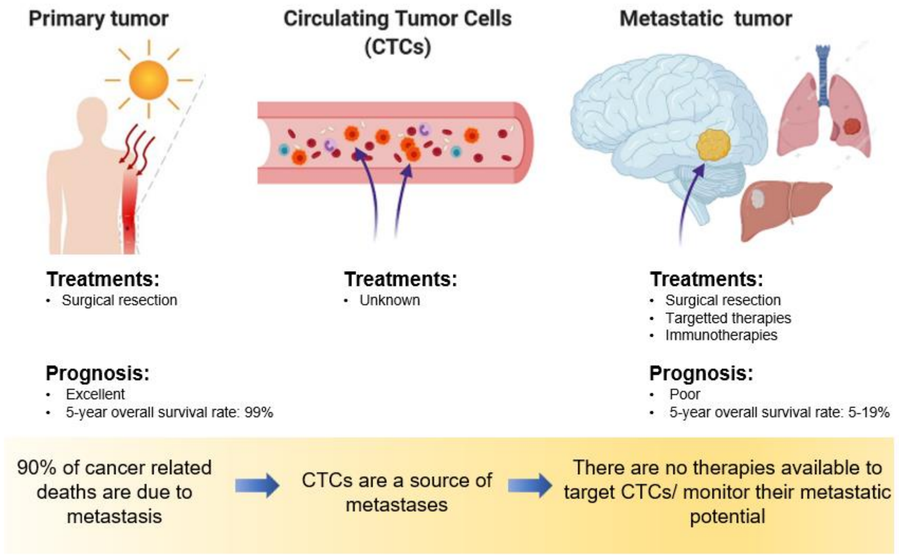


Figure 1: Schematic of metastatic spread to distant organs, treatment options and predicted prognosis.

- Currently, there are **no therapies targetting circulating tumour cells (CTCs)** which are the main source of metastatic tumours.
- CTCs represent an intermediate stage of the disease before the appearance of secondary tumours. High level of oxidative, physical and biochemical stress exerted by circulation leaves only a **few viable CTCs** which have **rewired their metabolism** to adapt, survive and colonize another site. Identification of these **metabolic changes** would open a new prognostic and therapeutic avenue.

OBJECTIVES

1) identify **novel metabolic genes/pathways** which enable survival of cancer cells in circulation and at secondary sites.
2) **validate** them using cell lines and in mouse models
3) use a **candidate gene approach** to test the role of **IDI1 gene** (mevalonate pathway) in CTC survival and metastasis

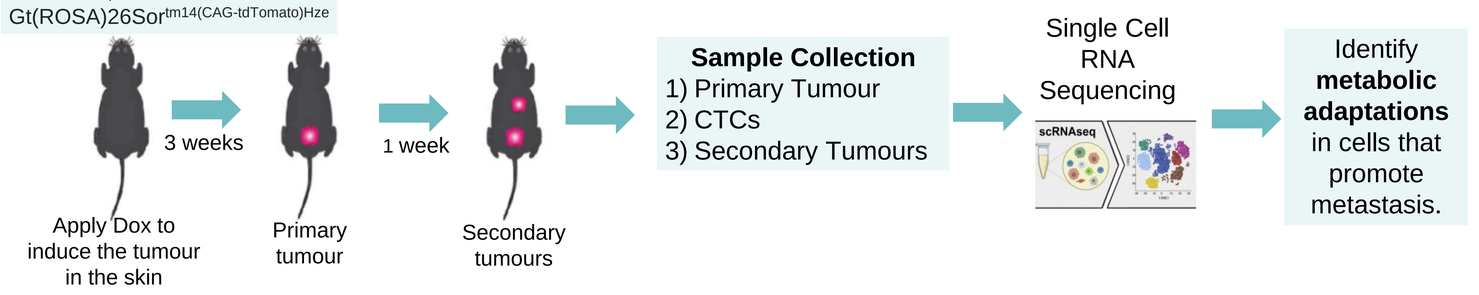
APPROACHES AND METHODOLOGY

1 UNBIASED DISCOVERY OF NOVEL METABOLIC GENES

A) Genetic Inducible Mouse Melanoma Model

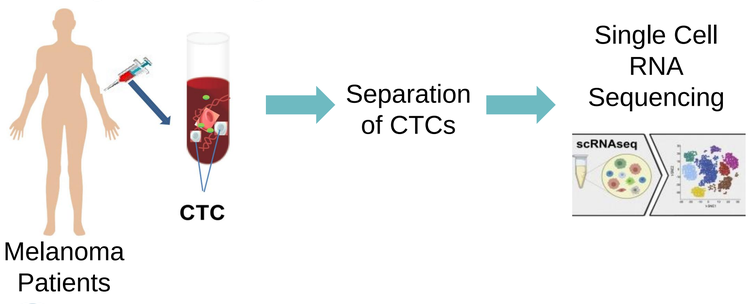
- Engineered with a similar genetic profile to human melanoma (BRAF-driven)
- Recapitulates the oncogenic events and disease progression seen in patients
- Tumour cells are fluorescently labelled to allow traceability.

Genotype
Tyr:CreER$^{(+)}$;
BRAF$^{CA(V600E)/CA(V600E)}$;
PTEN$^{lox/lox}$;
Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze}$

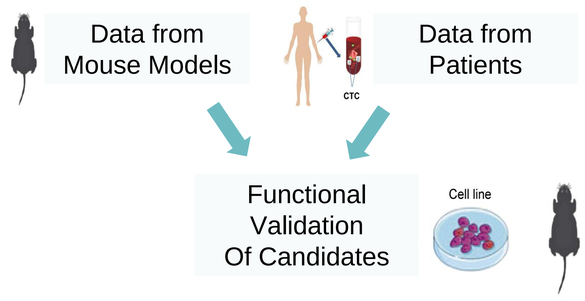


B) Patient Samples

- Single Cell RNA-sequencing on patient CTCs to integrate findings from the mouse model.

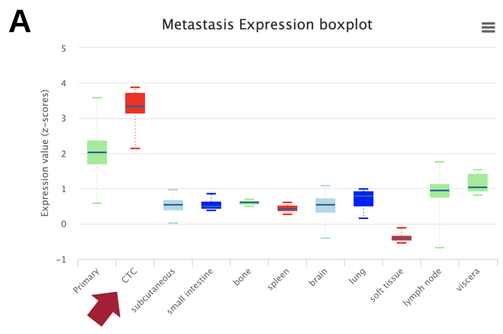


C) Functional Validation



2 CANDIDATE GENE APPROACH

- Mevanolate pathway provides important intermediates to cells for rapid growth and protection against oxidative stress.
- We hypothesise that the IDI1 branch of the pathway allows CTCs to survive in circulation and allow metastatic spreading.



B IDI1 over-expression/knock-down in human melanoma cells

- Phenotypic Analysis
- Xenographs in mice to test metastatic potential (survival of cells in circulation and spread to other organs)

Figure 2: A) Increased expression level of IDI1 in CTCs. B) Functional testing strategy.

PROGRESS AND PRELIMINARY DATA

1 Mouse Model

Optimising protocols for inducing tumours in the genetic model and tracing them in blood and at the metastatic sites.

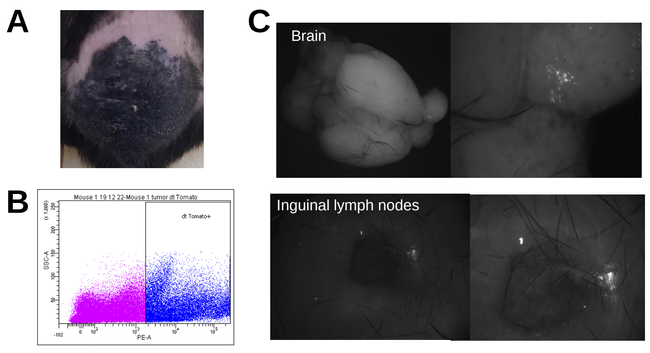


Figure 3: A) Primary tumour induced in the skin. B) Separation of the fluorescent cells to test the functionality of the fluorescent reporter. C) Investigating the metastatic sites using the fluorescent reporter.

2 Patient Samples

Isolation of CTCs from patient blood using a size-based separation system, independent of surface markers, to capture all the CTCs and the full heterogeneity among them.

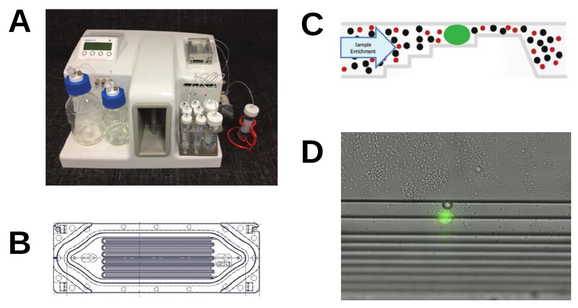


Figure 4: A) Parsortix: Size Based Cell Separation System B-C) Cell separation casette and mechanism of separation. D) Image of a captured cancer cell from patient blood.

3 *In vitro* studies on the candidate gene

Lentiviral over-expression/knock-down of the candidate gene (IDI1) for phenotypic analysis.

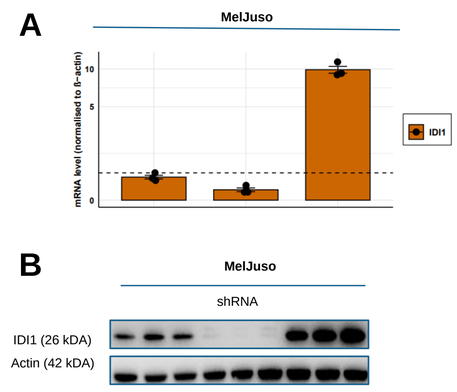


Figure 5: Confirmation of over-expression/knock-down of the candidate gene at A) mRNA level B) Protein level

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EXPECTED IMPACT

- Generate a landscape of transcriptomic changes during metastatic melanoma progression at a single cell resolution to foster discovery of **biomarkers** and **therapeutics.**
- Exploit **IDI1 as a potential marker** for disease progression and therapeutic target to interfere with metastasis.

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