Evolutionary dynamics of glucose-deprived cancer cells: Insights from experimentally-informed mathematical modelling

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Methodology

In vitro experiments

- We study the aggressive breast cancer cell line MCF7-sh-WISP2, obtained from the less aggressive MCF7 cell line.
- Glucose deprivation (GD) experiments (Exp): cells (cultured with 4.5g/l of glucose) are seeded in a medium initially containing 1g/l of glucose and monitored for 4-5 days.
- Rescue (R) Exp: as in GD Exp for 3 days, then the medium is changed to one initially containing 4.5g/l of glucose.
- MCT1 expression is analysed using flow cytometry.

Mathematical modelling

Key model quantities:

- Level of MCT1 expression: $y \in \mathbb{R}$.
- Cell population density function at time $t \geq 0$: $n(t,y) \geq 0$.
- Cell number $\rho(t)$, mean level of MCT1 expression $\mu(t)$ and related variance $\sigma^2(t)$:

$$\rho(t) = \int_{\mathbb{R}} n(t,y)\,\mathrm{d}y\,, \quad \mu(t) = \frac{1}{\rho(t)} \int_{\mathbb{R}} y\, n(t,y)\,\mathrm{d}y\,,$$
$$\sigma^2(t) = \frac{1}{\rho(t)} \int_{\mathbb{R}} y^2\, n(t,y)\,\mathrm{d}y\, - \mu(t)^2\,.$$

- Glucose and lactate concentrations: $G(t) \geq 0$, $L(t) \geq 0$.

Model equations:

- PDE for $n(t,y)$:

$$\frac{\partial n}{\partial t} = \underbrace{\Phi(G,L)\,\frac{\partial^2 n}{\partial y^2}}_{\substack{\text{spontaneous changes}\\ \text{in MCT1 expression}\\ \text{due to non-genetic}\\ \text{instability}}} - \underbrace{\left[\Psi^+(G,L) - \Psi^-(G,\mu)\right]\frac{\partial n}{\partial y}}_{\substack{\text{environment-induced}\\ \text{changes in MCT1}\\ \text{expression mediated}\\ \text{by lactate-associated}\\ \text{signalling pathways}}} \tag{1}$$
$$+ \Big[\, \underbrace{a(G,L)}_{\substack{\text{maximum}\\ \text{fitness}}} - \underbrace{b(G,L)}_{\substack{\text{strength}\\ \text{of natural}\\ \text{selection}}} \big(y - \underbrace{Y(G,L)}_{\substack{\text{fittest level}\\ \text{of MCT1}\\ \text{expression}}}\big)^2 - \underbrace{d\rho}_{\substack{\text{death due to}\\ \text{competition}\\ \text{for space}}} \,\Big]\, n\,.$$

- System of ordinary differential equations for the evolution of $G(t)$ and $L(t)$, coupled with (1).

Key assumptions:

- There is a threshold level of glucose $G^* > 0$ above which cells interrupt lactate uptake to prioritise glucose uptake.
- There is an MCT1 expression level y_L endowing cells with the highest rate of proliferation via glycolysis.
- There is an MCT1 expression level $y_H > y_L$ endowing cells with the highest rate of proliferation via lactate reuse when glucose is scarce (i.e. $G < G^*$).
- Then $y_L \leq Y(G,L) \leq y_H$, and for $G \geq G^*$

$$Y \equiv y_L\,, \quad \Psi^+ \equiv 0\,, \quad \Psi^- \propto (\mu - y_L)_+$$

while for $G < G^*$

$$\frac{\partial Y}{\partial L} > 0\,,\ \frac{\partial Y}{\partial G} < 0\,,\ \Psi^- \equiv 0\,,\ \frac{\partial \Psi^+}{\partial L} > 0\,,\ \frac{\partial \Phi}{\partial L} > 0\,.$$

Initial conditions (ICs):
We take

$$n(0,y) = \frac{\rho_0}{\sqrt{2\pi\sigma_0^2}} \exp\left(-\frac{(y-\mu_0)^2}{2\sigma_0^2}\right), \tag{2}$$

with ρ_0, μ_0, σ_0^2, $G(0)$ and $L(0)$ as in *in vitro* experiments.

Calibration and simulations

- The model and the data are nondimensionalised (ND).
- The set of ND data points is $\hat{S}_D = \{\hat{u}_D^i, i = 1, ..., M\}$.
- The set of ND parameter values is $\hat{S}_P \in \Omega \subset \mathbb{R}^N_{\geq 0}$. The corresponding summary statistics are $\{\hat{u}_P^i, i = 1, ..., M\}$.
- Assuming Gaussian measurement noise with zero mean, the likelihood of $\hat{S}_P$ is

$$\mathcal{L}(\hat{S}_P) = \mathbb{P}(\hat{S}_D \mid \hat{S}_P) = (2\pi\epsilon^2)^{-\frac{M}{2}} \prod_{i=1}^{M} \exp\left(-\frac{(\hat{u}_D^i - \hat{u}_P^i)^2}{2\epsilon^2}\right).$$

- The optimal parameter set (OPS) is obtained maximising $\mathcal{L}(\hat{S}_P)$, exploiting the in-built MATLAB function `bayesopt`.
- Bootstrapping is used for uncertainty quantification.
- Simulations are conducted solving the system of equations with a finite difference scheme (FTCS and upwind).

Summary

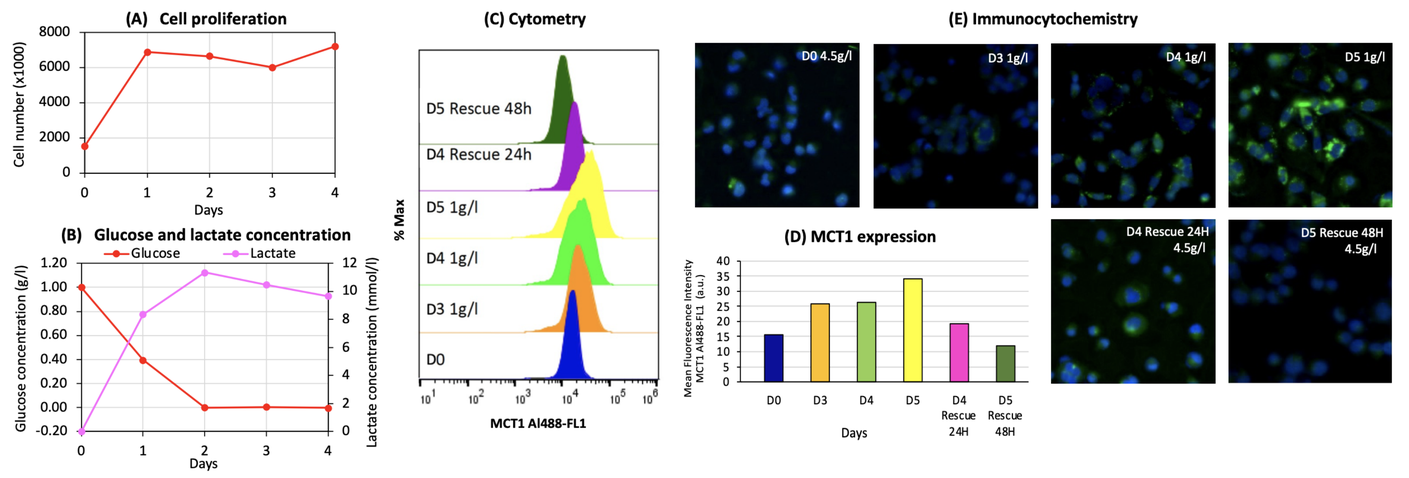
Background. Glucose is a primary energy source for cancer cells. Several lines of evidence support the idea that the overexpression of monocarboxylate transporters, such as MCT1 (a lactate cross-membrane transporter), mediates metabolic reprogramming of glucose-deprived aggressive cancer cells, allowing them to reuse lactate as an alternative energy source with serious consequences for disease progression. Nevertheless, the mechanisms underlying such a change in MCT1 expression remain, to this day, poorly explored.

Aim and approach. We employ a synergistic experimental and mathematical modelling approach to explore the evolutionary processes underlying the increase in MCT1 expression which mediates the adaptation of aggressive cancer cells to glucose deprivation. The results of *in vitro* experiments are used to inform and calibrate a mathematical model, which comprises a partial differential equation (PDE) for the dynamics of a cancer cell population structured by the MCT1 expression level.

Considered hypotheses. (i) Lactate may function as a signalling molecule, triggering regulatory pathways that modify the transcriptional activity of MCT1, thus mediating environment-induced changes in MCT1 expression. (ii) Spontaneous heritable and reversible changes in MCT1 expression may occur due to non-genetic instability, associated with gene expression noise (e.g. DNA methylation, histone modifications), which may be enhanced under glucose-deprivation.

Main results. Analytical and numerical results of the experimentally-calibrated model indicate that environment-induced changes in MCT1 expression enable a prompt adaptive response of glucose-deprived cancer cells, whilst spontaneous changes due to non-genetic instability create the substrate for environmental selection to act upon, speeding up the selective sweep underlying cancer cell adaptation, and may constitute a long-term bet-hedging mechanism.

Results from *in vitro* experiments



Dynamics of cell proliferation (A), glucose and lactate concentrations (B) in GD Exp. Dynamics of MCT1 expression in GD (Days D0, D3-D5) and R (D4, D5) Exp: MCT1 fluorescence intensity distributions (C), corresponding mean values (D) from flow cytometry analysis, and MCT1 antibody staining (green) from immunocytochemistry analysis (E).
Analogous GD Exp conducted on the breast cancer cell line MCF7 indicate sustained proliferation, lactate uptake and increase in MCT1 expression levels under GD are characteristic of aggressive tumours.

Results from the calibrated mathematical model

Proposition

PDE (1) subject to ICs (2), admits the exact solution

$$n(t,y) = \frac{\rho(t)}{\sqrt{2\pi\,\sigma^2(t)}} \exp\left[-\frac{(y-\mu(t))^2}{2\,\sigma^2(t)}\right],$$

with $\rho(t)$, $\mu(t)$ and $v(t) := 1/\sigma^2(t)$ being solutions to the Cauchy problem

$$\begin{cases} \dfrac{\mathrm{d}v}{\mathrm{d}t} = 2\left(b - \Phi v^2\right), \\ \dfrac{\mathrm{d}\mu}{\mathrm{d}t} = \dfrac{2\,b}{v}\,(Y - \mu) + \Psi^+ - \Psi^-, \\ \dfrac{\mathrm{d}\rho}{\mathrm{d}t} = \left[\left(a - \dfrac{b}{v} - b\,(Y-\mu)^2\right) - d\rho\right]\rho, \\ v(0) = 1/\sigma_0^2, \quad \mu(0) = \mu_0, \quad \rho(0) = \rho_0, \end{cases}$$

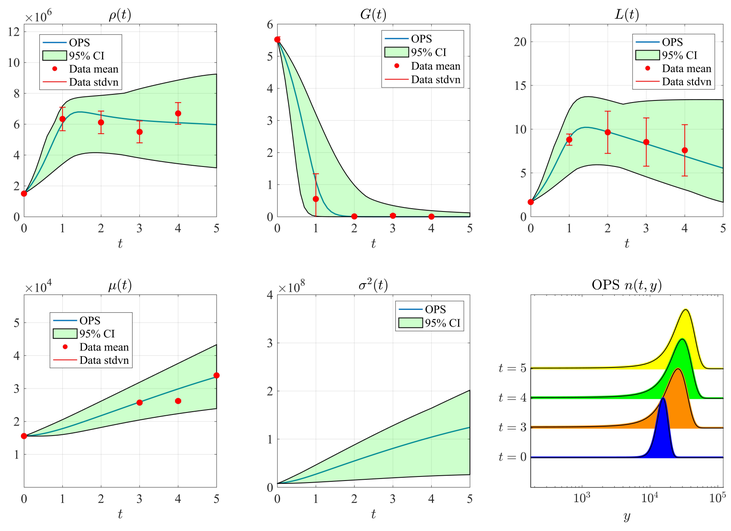
where a, b, Y, Φ and Ψ^+ are functions of (G,L) and Ψ^- of (G,μ). Moreover, under the additional assumptions $G(t) \equiv \overline{G} \geq 0$ and $L(t) \equiv \overline{L} \geq 0$, the solution is such that

$$\rho(t) \underset{t\to\infty}{\longrightarrow} \rho_\infty(\overline{G},\overline{L}), \quad \mu(t) \underset{t\to\infty}{\longrightarrow} \mu_\infty(\overline{G},\overline{L}),$$
$$\sigma^2(t) \underset{t\to\infty}{\longrightarrow} \sigma^2_\infty(\overline{G},\overline{L}),$$

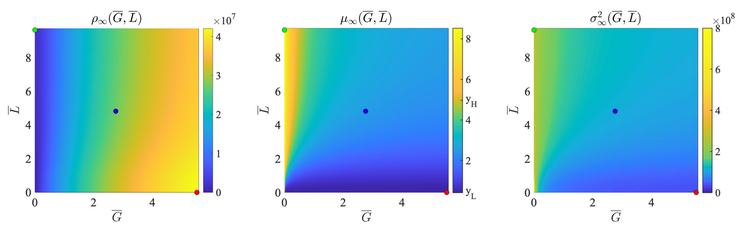
with

$$\rho_\infty = \max\left(0, \frac{1}{d}\left[a - \sqrt{\Phi b} - \frac{(\Psi^+)^2}{4\Phi}\right]\right),$$

$$\mu_\infty = Y + \frac{\Psi^+}{2\sqrt{\Phi b}}\,, \quad \sigma^2_\infty = \sqrt{\frac{\Phi}{b}}\,.$$



Simulation of GD Exp, under the OPS obtained from model calibration with experimental data.
Calibrating the model assuming $\Phi \equiv 0$ or $\Psi^\pm \equiv 0$, yields OPSs under- or over-estimating the variance.



Equilibrium values of the cell number ρ_∞, mean MCT1 expression level μ_∞ and related variance σ^2_∞ predicted by the calibrated mathematical model under constant concentrations of glucose and lactate.

Almeida et al., Journal of the Royal Society Interface, 21:20230587, 2024. Doi: 10.1098/rsif.2023.0587.