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

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Abstract

Glucose is a primary energy source for cancer cells. Several lines of evidence support the idea that monocarboxylate transporters, such as MCT1, elicit metabolic reprogramming of cancer cells in glucose-poor environments, allowing them to reuse lactate, a byproduct of glucose metabolism, as an alternative energy source with serious consequences for disease progression. We employ a synergistic experimental and mathematical modelling approach to explore the evolutionary processes at the root of cancer cell adaptation to glucose deprivation, with particular focus on the mechanisms underlying the increase in MCT1 expression observed in glucose-deprived aggressive cancer cells. Data from *in vitro* experiments on breast cancer cells are used to inform and calibrate a mathematical model that comprises a partial integro-differential equation for the dynamics of a population of cancer cells structured by the level of MCT1 expression. Analytical and numerical results of this model indicate that environment-induced changes in MCT1 expression mediated by lactate-associated signalling pathways 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 to glucose deprivation, and may constitute a long-term bet-hedging mechanism.

1 Introduction

Glucose is one of the primary nutrients used by cancer cells to produce energy, and glucose deficiency causes metabolic stress, cell dysfunction, and eventual death [1]. In fact, glucose consumption not only results in decreased nutrient availability, but also generally correlates with lactate production and the development of an acidic extracellular environment [2–4]. Cancer cells can rely on a variety of mechanisms that activate protective functions under metabolic and environmental stress, including metabolic reprogramming [5]. Accumulating evidence indicates that aggressive cancer cells may acquire the ability to absorb lactic acid and use it to synthesise pyruvate [3, 6, 7], thus converting harmful byproducts of glucose metabolism into alternative energy sources. Lactic acid is transported across cell membranes through a family of four reversible monocarboxylate transporters (MCTs) belonging to the SLC16/MCT family of solute carriers [8]. Amongst these, MCT1 is the most widely expressed and facilitates lactate and pyruvate upload [9, 10]. Such an increase in pyruvate and lactate metabolism has been associated with enhanced invasion and migration, and higher survival in the circulation, with overall consequences

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for metastasis [11,12], to the extent that MCT1 inhibition has been investigated as a potential therapeutic target [13,14].

While it is evident that the overexpression of MCT1 plays a key role in the metabolic reprogramming of glucose-deprived aggressive cancer cells, allowing them to reuse lactate as an alternative energy source with serious consequences for disease progression, the mechanisms underlying such a change in MCT1 expression remain, to this day, poorly explored. On the one hand, lactate may function as a signalling molecule, triggering regulatory pathways that modify the transcriptional activity of MCT1 [6,15], thus mediating environment-induced changes in MCT1 expression [16]. On the other hand, cancer cells are known to undergo spontaneous heritable and reversible changes in gene expression due to non-genetic instability [17,18]. This is generally associated with noise in gene expression and may be due, for instance, to DNA methylation or histone modifications [19,20] occurring over the lifespan of a tumour cell. As lactate has been shown to be responsible for certain histone modifications [21,22], the effect of non-genetic instability may even be enhanced under glucose-deprivation.

In this work, a synergistic experimental and mathematical modelling approach is employed to explore the evolutionary processes at the root of cancer cell adaptation to glucose deprivation, with particular focus on the mechanisms underlying the increase in MCT1 expression observed in glucose-deprived aggressive cancer cells. Data from *in vitro* experiments on breast cancer cells, which were specifically carried out for this study, are used to inform a mathematical model that comprises a partial integro-differential equation (PIDE) for the dynamics of a population of cancer cells structured by the level of MCT1 expression, which is coupled with a system of ordinary differential equations (ODEs) for the dynamics of glucose and lactate present in the extracellular environment. This model allows for predictions on the dynamics of the MCT1 expression distribution of cancer cells to be made and to be directly compared with the results of flow cytometry analyses, while making it also possible to dissect out the evolutionary processes underlying these dynamics. Related mathematical models have been employed to investigate cancer cell adaptation to hypoxia [23–30], but not to assess MCT1-associated changes in lactate uptake. Furthermore, alternative mathematical models have been proposed to study the role of MCT1 expression-regulated lactate uptake in the coexistence of different metabolic pathways within the same tumour [4,31,32], but none of these proposes a causal mechanism for the reported increase in MCT1 expression.

Experimental data are used to carry out model calibration, through a likelihood-maximising method [33–35], and the results of numerical simulations of the calibrated model are complemented with analytical results on the qualitative and quantitative properties of the solution to the PIDE that governs the evolution of cancer cells. These results shed light on the evolutionary dynamics of glucose-deprived cancer cells by elucidating the respective roles that environment-induced changes in MCT1 expression mediated by lactate-associated signalling pathways and spontaneous changes due to non-genetic instability play in the adaptation of cancer cell populations to glucose-poor environments.

2 Methods

2.1 *In vitro* experiments

A summary of the experimental set-up is provided below, and full details of experimental materials and methods can be found in Sup.Mat.S2.

Cell lines

Breast cancer cells of the MCF7 and MCF7-sh-WISP2 lines are considered, with the latter being obtained from the former upon inducing epithelial-to-mesenchymal transition through WISP2 gene silencing. The focus of this study is on the MCF7-sh-WISP2 cell line, which has been documented to be more invasive and aggressive than the MCF7 cell line [36,37]. We also report on the results from experiments conducted on MCF7 cells to corroborate the hypothesis that fast metabolic reprogramming associated with lactate uptake under glucose deprivation is characteristic of more aggressive cancer cells.

‘Glucose-deprivation’ and ‘rescue’ experiments

Cells originally maintained in medium containing 4.5g/l of glucose (i.e. a high level of glucose) are seeded, at high cell numbers, in a medium containing 1g/l of glucose (i.e. a physiological level of glucose). In ‘glucose-deprivation’ experiments, glucose is not re-added to the medium during cell culture (i.e. there is no glucose replenishment) so that, due to consumption by the cells, glucose levels drop during the course of the experiment and thus cells experience glucose deprivation. In ‘rescue’ experiments, a similar protocol is followed for a few days and the culture medium is subsequently changed to a medium containing 4.5g/l of glucose, where cells are cultured for a few more days, so that cells first experience glucose deprivation and are then rescued from it.

Measured quantities

Over the span of several days, we tracked: viable cell numbers and percentages of apoptotic cells; glucose and lactate concentrations in the cell culture medium; MCT1 expression distribution (i.e. fluorescence-intensity distributions), obtained through flow cytometry analysis and complemented with images from immunocytochemistry analysis; mRNA expression of different MCT proteins measured by RT-qPCR – i.e. MCT1, MCT2 (an MCT very similar to MCT1 although it displays a higher affinity for L-lactic acid and pyruvate), and MCT4 (an efficient lactate exporter expressed in glycolytic cells that is not required for lactate uptake [9, 10]).

2.2 Mathematical modelling

Building on the modelling strategies presented in [38, 39], we develop a mathematical model that describes the evolutionary dynamics of a population of MCF7-sh-WISP2 cells, structured by the level of MCT1 expression, under the environmental conditions which are determined by the levels of glucose and lactate in the extracellular environment. An outline of the model is provided below, while a detailed description of the model equations alongside the main modelling assumptions, which are informed by the results of *in vitro* experiments underlying this study, is provided in Sup.Mat.S1.

Key model quantities

The model comprises a PIDE for the dynamics of the cell population density function $n(t, y)$, which represents the number of MCF7-sh-WISP2 cells with level of MCT1 expression $y \in \mathbb{R}$ at time $t \in \mathbb{R}_+$ (i.e. the MCT1 expression distribution of MCF7-sh-WISP2 cells at time t). Such a PIDE is coupled with a system of ODEs for the dynamics of the concentrations of glucose and lactate in the extracellular environment $G(t)$ and $L(t)$. The cell number, the mean level of MCT1 expression and the related variance, which provides a possible measure for the level of intercellular variability in MCT1 expression, are computed, respectively, as

$$\rho(t) = \int_{\mathbb{R}} n(t, y) dy, \quad \mu(t) = \frac{1}{\rho(t)} \int_{\mathbb{R}} y n(t, y) dy, \quad \sigma^2(t) = \frac{1}{\rho(t)} \int_{\mathbb{R}} y^2 n(t, y) dy - \mu^2(t). \quad (2.1)$$

Modelling cell proliferation and death under environmental selection on MCT1 expression

The results of *in vitro* experiments (cf. Sec. 3.2) indicate that the MCT1 expression distribution of MCF7-sh-WISP2 cells is, to a first approximation, unimodal with a single peak at the centre of the distribution. The location of the centre of the distribution moves from lower to higher expression levels when cells experience glucose deprivation and from higher to lower expression levels when cells are rescued from glucose deprivation. Hence, we assume that there is a level of MCT1 expression (i.e. the fittest level of MCT1 expression) endowing cells with the highest fitness depending on the environmental conditions determined by the concentrations of glucose and lactate. Moreover, the results of *in vitro* experiments (cf. Sec. 3.1) support the idea that proliferation and survival of MCF7-sh-WISP2 cells correlate with glucose uptake when glucose levels are sufficiently high and with lactate uptake when glucose levels are

low. Therefore, we further assume that there are a level of MCT1 expression, y_L , endowing cells with the highest rate of proliferation via glycolysis and a higher level of MCT1 expression, $y_H > y_L$, endowing cells with the highest rate of proliferation via lactate reuse when glucose is scarce – i.e. if the concentration of glucose in the extracellular environment is lower than a threshold level G^* above which cells stop taking lactate from the extracellular environment in order to prioritise glucose uptake. Under these assumptions, in the framework of our model, the fittest level of MCT1 expression is represented by the function $Y(G, L)$, defined via Eq. (S.14) in Sup.Mat.S1, which is such that if $G \geq G^*$ then $Y(G, L) = y_L$ for any $L \geq 0$, whereas if $G < G^*$ then $Y(G, L) \rightarrow y_H$ as G decreases and L increases. Furthermore, the strength of environmental selection on MCT1 expression is linked to the value of the selection gradient $b(G, L)$ defined via Eq. (S.12) in Sup.Mat.S1,

Modelling spontaneous and environment-induced changes in MCT1 expression

The effects of changes in the level of cell expression of MCT1 are also incorporated into the model. In particular, we let spontaneous changes due to non-genetic instability occur at rate Φ . Moreover, we assume that environment-induced changes mediated by lactate-associated signalling pathways lead to an increase in MCT1 expression at rate Ψ^+ under glucose deprivation (i.e. when $G < G^*$) and to a decrease in MCT1 expression at rate Ψ^- when the glucose level is sufficiently high (i.e. when $G \geq G^*$).

2.3 Model calibration based on experimental data

Experimental data on MCF7-sh-WISP2 cells are used to carry out model calibration through a likelihood-maximising method [33–35]. In summary, the mathematical model and the data are first nondimensionalised to avoid calibration bias. Then the optimal parameter set (OPS) is obtained, through an iterative process, by maximising the likelihood, defined assuming Gaussian measurement noise with zero mean, exploiting the in-built MATLAB function `bayesopt`, which is based on Bayesian Optimisation. At each iteration, we solve numerically the PIDE-ODE system that constitutes the model, using the methods described in Sup.Mat.S2. The MATLAB source code along with the data used for model calibration have been made available on GitHub¹. In order to explore a variety of evolutionary scenarios, calibration was carried out for the model in which both spontaneous and environment-induced changes in MCT1 expression are included (i.e. $\Phi \neq 0$ and $\Psi^\pm \neq 0$), and for reduced models that take into account only spontaneous (i.e. $\Phi \neq 0$ and $\Psi^\pm \equiv 0$) or environment-induced (i.e. $\Phi \equiv 0$ and $\Psi^\pm \neq 0$) changes. The obtained OPSs are reported in Tab.S1 in Sup.Mat.S2.

2.4 Simulation and analysis of the model

To explore the mechanisms underlying the evolutionary dynamics of MCF7-sh-WISP2 cells under glucose deprivation, the results of numerical simulations of the calibrated model, which are carried out using the numerical methods described in Sup.Mat.S2.4, are integrated with the analytical results presented in Sup.Mat.S2.5, which build on the results presented in [30, 40–43].

3 Main results

3.1 Proliferation and survival of MCF7-sh-WISP2 cells correlate with lactate uptake under glucose deprivation

Fig.1 and Fig.S1 in Sup.Mat.S3 summarise the dynamics of cell proliferation and glucose and lactate concentrations in the cell culture medium observed during ‘glucose-deprivation’ experiments conducted for four days on MCF7-sh-WISP2 and MCF7 cells, respectively. The corresponding dynamics of cell death are summarised by Fig.S2 in Sup.Mat.S3. These results demonstrate that there is a stark difference in the proliferation dynamics of the MCF7-sh-WISP2 and MCF7 cell lines under glucose deprivation, with

¹<https://github.com/ChiaraVilla/AlmeidaEtAl2023Evolutionary>

the former reaching numbers of viable cells over twice as high as the latter. Moreover, cell death in the MCF7-sh-WISP2 line does not significantly increase over time, as opposed to the MCF7 line for which the percentage of apoptotic cells undergoes a four-fold increase during the experiment. The dynamics of the concentration of glucose in the culture medium of the two cell lines are similar, though only cells of the MCF7-sh-WISP2 line consume all the glucose available. Furthermore, the concentration of lactate in the culture medium of MCF7 cells displays a steady increase mirroring glucose consumption, whilst a decline in lactate concentration in the culture medium of MCF7-sh-WISP2 cells is observed when little to no glucose is present in the medium, thus suggesting that lactate uptake occurs amongst MCF7-sh-WISP2 cells under glucose deprivation. Taken together, these experimental results support the idea that proliferation and survival of MCF7-sh-WISP2 cells correlate with glucose consumption when glucose levels are sufficiently high and with lactate uptake under glucose deprivation.

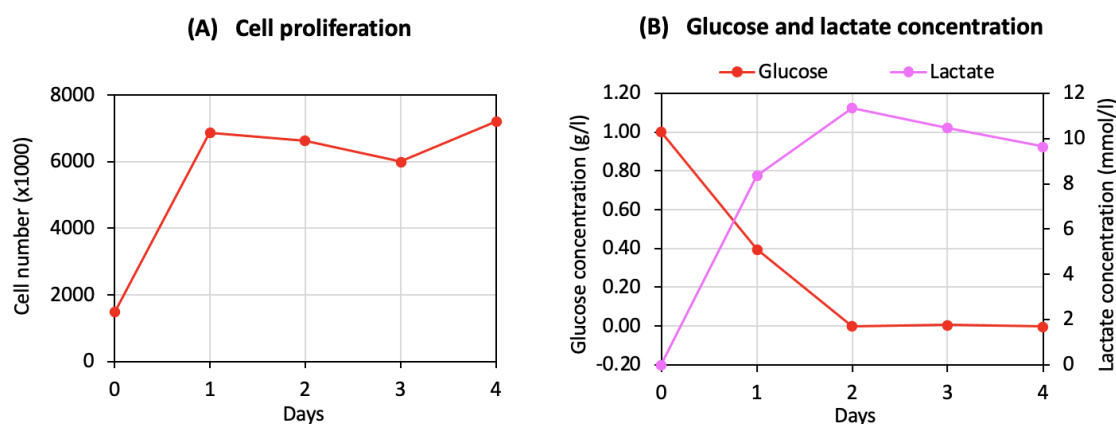


Figure 1: **Dynamics of cell proliferation and glucose and lactate concentrations in ‘glucose-deprivation’ experiments conducted on MCF7-sh-WISP2 cells.** Dynamics of cell proliferation (panel (A)), glucose concentration (panel (B), red line, left y-axis) and lactate concentration (panel (B), pink line, right y-axis) in ‘glucose-deprivation’ experiments conducted on MCF7-sh-WISP2 cells for four days. Cell proliferation was assessed by counting the number of viable cells upon seeding (i.e. day 0) and at the end of each day of culture (i.e. days 1-4). Glucose and lactate concentrations were measured in the cell culture medium on days 0-4.

3.2 Glucose deprivation induces a reversible increase in MCT1 expression of MCF7-sh-WISP2 cells

The experimental results summarised by Fig.2 show a steady increase in MCT1 expression of MCF7-sh-WISP2 cells throughout ‘glucose-deprivation’ experiments. On the other hand, in ‘rescue’ experiments, MCT1 expression levels of MCF7-sh-WISP2 cells increase during the glucose-deprivation phase of the experiment and then decrease again during the phase of rescue from glucose deprivation, which demonstrates reversibility of changes in MCT1 expression. Similar trends are observed in the MCT1 mRNA levels of MCF7-sh-WISP2 cells during ‘glucose-deprivation’ and ‘rescue’ experiments, whereas an increase resembling the one detected in the MCT1 protein expression levels is not observed in the MCT2 mRNA levels, and no MCT4 mRNA is detected (cf. Fig.S4 in Sup.Mat.S3). In contrast, no appreciable change in MCT1 expression of MCF7 cells is observed during both ‘glucose-deprivation’ and ‘rescue’ experiments (cf. Fig.S3 in Sup.Mat.S3).

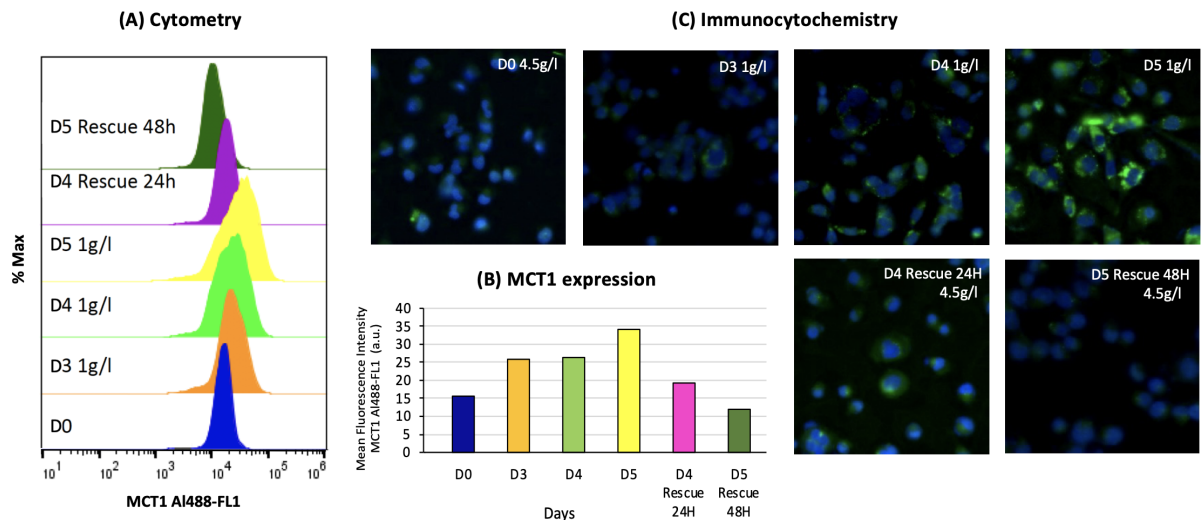


Figure 2: **Dynamics of MCT1 expression in ‘glucose-deprivation’ and ‘rescue’ experiments conducted on MCF7-sh-WISP2 cells.** (A),(C) MCT1 protein expression of MCF7-sh-WISP2 cells, assessed through flow cytometry analysis (panel (A)) and immunocytochemistry analysis using an MCT1 antibody (green staining in panel (C)), upon seeding (i.e. on day 0) and on days 3-5 of ‘glucose-deprivation’ experiments conducted for five days (sub-panel D0 and sub-panels D3-D5). MCT1 protein expression of MCF7-sh-WISP2 cells during the phase of rescue from glucose deprivation in the corresponding ‘rescue’ experiments (i.e. on days 4 and 5) is also displayed (sub-panels D4 Rescue and D5 Rescue). (B) Mean fluorescence intensity of MCT1 labelling for MCF7-sh-WISP2 cells (in units of 10^3), obtained from the fluorescence intensity distributions of panel (A) plotted on a logarithmic scale.

3.3 Both spontaneous and environment-induced MCT1-expression changes contribute to the adaptation of MCF7-sh-WISP2 cells to glucose deprivation

There is good quantitative agreement between numerical results obtained by simulating ‘glucose-deprivation’ and ‘rescue’ experiments through the calibrated model in which both spontaneous and environment-induced changes in MCT1 expression are included (i.e. $\Phi \neq 0$ and $\Psi^\pm \neq 0$) and experimental observations for MCF7-sh-WISP2 cells deprived of glucose and rescued from glucose deprivation, as shown by the plots in Fig.3, and as additionally highlighted by the high likelihood associated with the obtained OPS (cf. Tab.S1 in Sup.Mat.S2). Instead, levels of intercellular variability in MCT1 expression much higher or lower than those estimated from experimental data are observed in numerical simulations of the experiments carried out through calibrated reduced models that take into account only spontaneous or environment-induced changes in MCT1 expression (i.e. $\Phi \neq 0$ and $\Psi^\pm \equiv 0$ or $\Phi \equiv 0$ and $\Psi^\pm \neq 0$), respectively, as demonstrated by the dynamics of the variance of the MCT1 expression distribution, σ^2 , displayed in Fig.S5(d) in Sup.Mat.S3. Furthermore, the results of numerically simulated ‘glucose-deprivation’ experiments carried out over a time span longer than that of *in vitro* experiments suggest that the synergy between these two forms of changes in MCT1 expression accelerates collective cell adaptation to glucose deprivation, as demonstrated by the fact that, when $\Phi \neq 0$ and $\Psi^\pm \neq 0$, the mean level of MCT1 expression, μ , converges more quickly to the level y_H , which in our modelling framework is the level endowing MCF7-sh-WISP2 cells with the maximum capability of taking lactate from the extracellular environment and reusing it to produce the energy required for their proliferation when glucose is scarce (cf. Fig.S6 in Sup.Mat.S3).

Taken together, these results support the idea that both spontaneous and environment-induced changes in MCT1 expression contribute to the adaptation of MCF7-sh-WISP2 cells to glucose deprivation. In

particular, the modelling assumptions underlying these numerical results provide the following theoretical explanation for the increase in the mean level of MCT1 expression experimentally observed amongst glucose-deprived MCF7-sh-WISP2 cells. Cells with different levels of MCT1 expression emerge as a consequence of spontaneous changes in gene expression due to non-genetic instability. On top of this, as the glucose concentration decreases and the lactate concentration increases during ‘glucose-deprivation’ experiments, environment-induced changes in gene expression mediated by lactate-associated signalling pathways lead cells to express MCT1 at a higher level. Cells with levels of MCT1 expression closer to the fittest one, which in glucose-poor environments is higher than in glucose-rich environments, are then dynamically selected. The interplay between these evolutionary processes results in a progressive increase in the mean level of MCT1 expression of MCF7-sh-WISP2 cells.

3.4 Respective contributions of spontaneous and environment-induced MCT1-expression changes in the adaptation of MCF7-sh-WISP2 cells to glucose deprivation

The analytical results of Proposition S2.1 in Sup.Mat.S2 (cf. Eqs. (S28)₁ and (S28)₂ along with the relations given by Eq.(S4)) clarify how spontaneous and environment-induced changes in gene expression, along with environmental selection on MCT1 expression, affect the dynamics of the mean level of MCT1 expression, μ , and the corresponding variance, σ^2 , in MCF7-sh-WISP2 cells. In summary, larger values of the rate of spontaneous changes in MCT1 expression, Φ , accelerate the growth of σ^2 , while a stronger environmental selection on MCT1 expression (i.e. a larger selection gradient b) leads to reduced values of σ^2 . In turn, larger values of σ^2 enhance the rate at which μ approaches the fittest level of MCT1 expression, Y . Such a rate also increase with the strength of environmental selection on MCT1 expression (i.e. the selection gradient b). Moreover, under glucose deprivation, larger values of the rate at which environment-induced changes lead to an increase in MCT1 expression, Ψ^+ , promote the growth of μ . These analytical results are confirmed by the results of numerical simulations of ‘glucose-deprivation’ experiments presented in Fig.S7 in Sup.Mat.S3, which show that larger values of Φ and Ψ^+ correlate with a faster increase of σ^2 and μ .

Taken together, these results clarify the roles played by spontaneous and environment-induced changes in MCT1 expression in the evolutionary dynamics of glucose-deprived MCF7-sh-WISP2 cells. The former promote intercellular variability in MCT1 expression, which creates the substrate for environmental selection to act upon and speed up the selective sweep underlying collective cell adaptation to glucose deprivation, while the latter triggers a prompt adaptive response of glucose-deprived MCF7-sh-WISP2 cells by promoting overexpression of MCT1. These conclusions are also supported by the fact that estimation of the model parameters from experimental data (cf. the OPS reported in Tab.S1 in Sup.Mat.S2) indicate that the rate of environment-induced changes in the level of MCT1 expression is approximately three orders of magnitude larger than the rate of spontaneous changes.

3.5 Spontaneous MCT1-expression changes may constitute a long-term bet-hedging mechanism for MCF7-sh-WISP2 cells under glucose deprivation

The mathematical model makes it possible to explore the cell evolutionary dynamics beyond timescales and scenarios which can be investigated through experiments. In particular, the analytical results of Theorem S2.2 in Sup.Mat.S2 (cf. Remark S2.3 in Sup.Mat.S2) provide a complete characterisation of the equilibrium values of the number, the mean level of MCT1 expression and the related variance of MCF7-sh-WISP2 cells under virtual scenarios where the glucose and lactate concentrations are kept constant, i.e. a complete characterisation of the limits $\rho(t) \rightarrow \rho_\infty$, $\mu(t) \rightarrow \mu_\infty$ and $\sigma^2(t) \rightarrow \sigma_\infty^2$ as $t \rightarrow \infty$ when $G(t) = \bar{G}$ and $L(t) = \bar{L}$ for all $t \geq 0$. These analytical results are confirmed by the results of numerical simulations of the calibrated model summarised by Fig.S8 in Sup.Mat.S3, which demonstrate that the equilibrium values ρ_∞ , μ_∞ and σ_∞^2 are ultimately attained when $(G(t), L(t)) \equiv (\bar{G}, \bar{L})$.

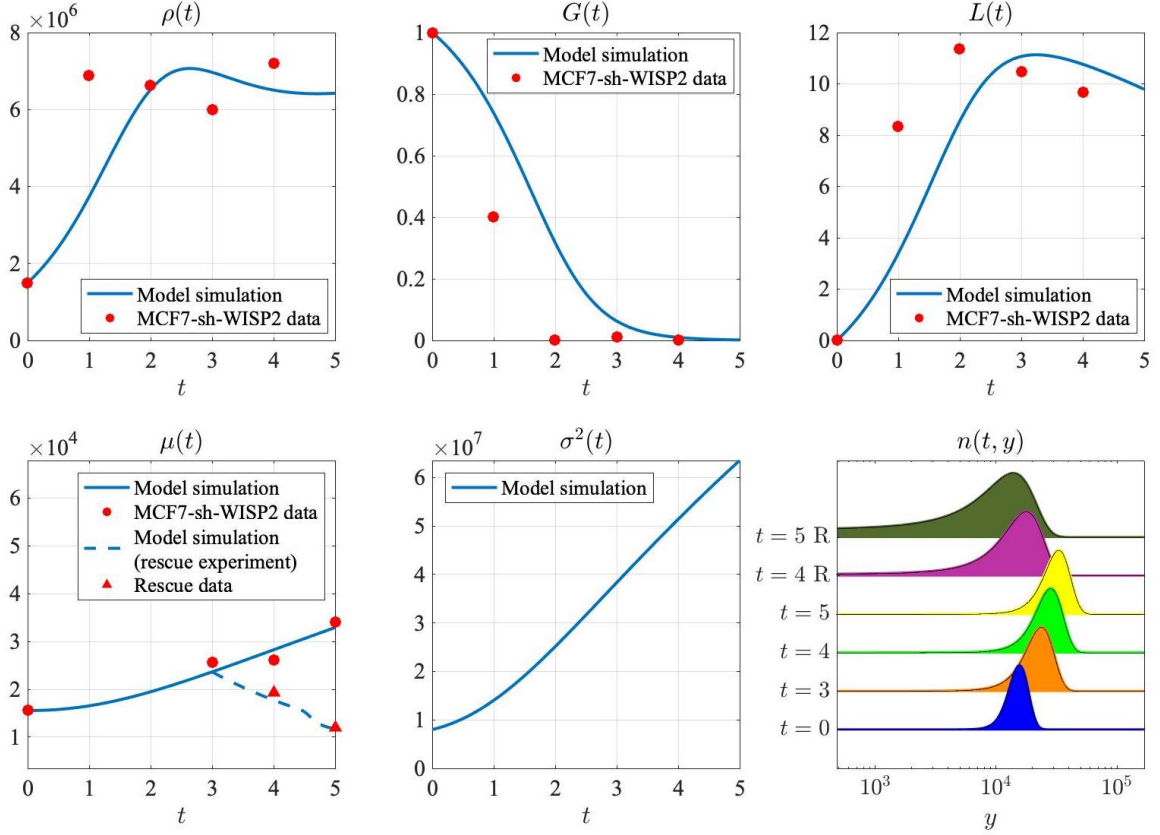


Figure 3: **Numerical simulations of ‘glucose-deprivation’ and ‘rescue’ experiments conducted on MCF7-sh-WISP2 cells.** Simulated dynamics of the cell number $\rho(t)$ (top-left panel), the glucose concentration $G(t)$ (top-central panel), the lactate concentration $L(t)$ (top-right panel), the mean level of MCT1 expression $\mu(t)$ (bottom-left panel, solid line), the related variance $\sigma^2(t)$ (bottom-central panel), and the MCT1 expression distribution $n(t, y)$ (bottom-right panel, $t = 0 - t = 5$) in ‘glucose-deprivation’ experiments conducted on MCF7-sh-WISP2 cells. Numerical simulations were carried out for the calibrated model in which both spontaneous and environment-induced changes in MCT1 expression are included (i.e. $\Phi \neq 0$ and $\Psi^\pm \neq 0$), under the OPS reported in Tab.S1 in Sup.Mat.S2. The MCT1 expression distribution is plotted on the logarithmic scale as for the outputs of flow cytometry analyses to facilitate visual comparison. The MCT1 expression distribution during the phase of rescue from glucose deprivation in the corresponding simulations of ‘rescue’ experiments is also displayed (bottom-right panel, $t = 4$ R and $t = 5$ R) along with the mean level of MCT1 expression (bottom-left panel, dashed line). The red markers highlight experimental data that are used to carry out model calibration, with circles and triangles corresponding to ‘glucose-deprivation’ and ‘rescue’ experiments, respectively. The values of t are in days, the values of $G(t)$ are in g/l, and the values of $L(t)$ are in mmol/l.

The results of Theorem S2.2 complement the results discussed in Sec. 3.3 by showing that the equilibrium value of the variance of the MCT1 expression distribution, σ_∞^2 , increases with the rate of spontaneous changes in MCT1 expression, Φ , and decreases with the strength of environmental selection on MCT1 expression (i.e. the selection gradient b). The results of Theorem S2.2 also demonstrate that when glucose is scarce and lactate is present (i.e. when $\bar{G} < G^*$ and $\bar{L} > 0$), and thus under glucose deprivation, the distance between the equilibrium value of the mean level of MCT1 expression, μ_∞ , and the fittest level of MCT1 expression, Y , increases with the rate at which environment-induced changes lead to an increase in MCT1 expression, Ψ^+ , and decreases with both the rate of spontaneous changes, Φ , and the strength of

environmental selection on MCT1 expression (i.e. the selection gradient b). This supports the idea that, whilst enabling a faster adaptive response to glucose deprivation, as discussed in Sec. 3.4, environment-induced changes in MCT1 expression may ultimately lead to suboptimal adaptation, whereas spontaneous changes may constitute a long-term bet-hedging mechanism.

Moreover, the heat maps in Fig. 4 illustrate how the equilibrium values ρ_∞ , μ_∞ and σ_∞^2 vary with the glucose and lactate concentrations \bar{G} and \bar{L} , under the OPS reported in Tab.S1 in Sup.Mat.S2. In summary, when glucose is scarce (i.e. for $\bar{G} < G^*$ with $G^* \approx 1\text{g/l}$ in the obtained OPS): μ_∞ decreases with \bar{G} and increases with \bar{L} ; σ_∞^2 increases as \bar{G} decreases and reaches maximum levels when \bar{L} is also small; ρ_∞ increases with both \bar{G} and \bar{L} . On the other hand, when the glucose level is sufficiently high (i.e. for $\bar{G} \geq G^*$), μ_∞ drops to y_L and both ρ_∞ and σ_∞^2 vary very little with \bar{G} and \bar{L} – i.e. ρ_∞ remains relatively high and σ_∞^2 remains relatively low.

These findings recapitulate the results of numerical simulations of ‘glucose-deprivation’ and ‘rescue’ experiments displayed in Fig.3 by corroborating the idea that, whereas lower mean levels of MCT1 expression emerge when the concentration of glucose in the extracellular environment is sufficiently high, as in ‘rescue’ experiments, glucose deprivation leads to the selection for cells that are capable of exploiting lactate as an alternative energy source, which results in higher mean levels of MCT1 expression amongst MCF7-sh-WISP2 cells and allows for relatively high cell numbers in spite of glucose scarcity.

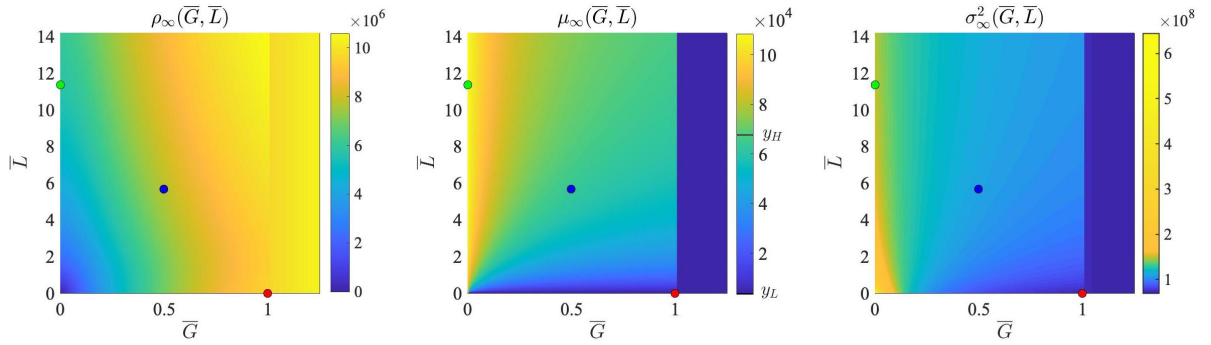


Figure 4: **Equilibrium values of the number, the mean level of MCT1 expression and the related variance of MCF7-sh-WISP2 cells predicted by the mathematical model under constant concentrations of glucose and lactate.** Plots of the equilibrium number ρ_∞ (left panel), the mean level of MCT1 expression μ_∞ (central panel), and the related variance σ_∞^2 (right panel) of MCF7-sh-WISP2 cells given by Theorem S2.2 in Sup.Mat.S2 (cf. Eq.(S51)) as functions of constant concentrations of glucose and lactate (\bar{G}, \bar{L}), under the OPS reported in Tab.S1 in Sup.Mat.S2. The green, blue and red dots highlight the values of $(G(t), L(t)) \equiv (\bar{G}, \bar{L})$ that are used to obtain the numerical results of Fig.S8 in Sup.Mat.S3. The values of \bar{G} are in units of g/l, while the values of \bar{L} are in units of mmol/l.

4 Discussion

We adopted an experimentally-informed mathematical modelling approach to investigate the evolutionary dynamics of glucose-deprived cancer cells.

In vitro experiments were conducted on two breast cancer cell lines, MCF7 and MCF7-sh-WISP2, seeded at high cell numbers and quickly consuming the glucose initially available at physiological levels. Experimental outputs revealed that the more aggressive MCF7-sh-WISP2 cells have the ability to survive and sustain substantial proliferation in low-glucose conditions, as opposed to the less aggressive MCF7 cells. Changes in lactate levels *in situ* suggested lactate uptake by MCF7-sh-WISP2 cells, and flow cytometry and immunocytochemistry analyses indicated an associated increase in MCT1 expression, which was then reversed when cells were rescued and exposed again to higher glucose levels.

Experimental data on the MCF7-sh-WISP2 cell line were used to calibrate the proposed mathematical model of cell evolutionary dynamics, and the MCT1 expression distributions obtained through flow cytometry analyses were compared with those predicted by the mathematical model. We found that the calibrated model, whose numerical simulation results are in good quantitative agreement with experimental data, best reproduces experimental observations when the effects of both spontaneous and environment-induced changes in MCT1 expression are taken into account. This finding indicates that cognate studies considering only one of these two types of changes in gene expression may be overestimating the rates at which the considered type of change occurs, overall disregarding the combined effect of the two of them.

The analytical and numerical results of the calibrated model presented here indicate that environment-induced changes in MCT1 expression mediated by lactate-associated signalling pathways enable a prompt adaptive response of glucose-deprived cancer cells. Furthermore, spontaneous changes in MCT1 expression due to non-genetic instability create the substrate for natural selection to act upon, speeding up the selective sweep underlying cancer cell adaptation to glucose deprivation, and may constitute a long-term bet-hedging mechanism. These results on the respective roles played by spontaneous and environment-induced changes in gene expression in the evolutionary dynamics of cancer cells, whilst having been obtained for glucose-deprived cells of the MCF7-sh-WISP2 line, may extend to other cell lines and scenarios whereby changes in gene expression elicit metabolic reprogramming of cancer cells under nutrient deprivation – e.g. HIF1 favouring anaerobic energy pathways or CD36 promoting fatty acid uptake [11, 29, 44].

The optimal parameter set obtained from model calibration suggests that the MCT1 fluorescence intensity levels recorded at the end of the *in vitro* experiments on MCF7-sh-WISP2 cells do not correspond to maximal levels of lactate uptake, and MCT1 expression levels may continue to increase over the span of a month. In practice, performing the experiments over a longer timeframe we expect cells to die out faster than as predicted by the model, due to external factors, demographic stochasticity at low cell numbers or additional byproducts of cell metabolism that are not incorporated into the modelling framework proposed here. Nevertheless, it is evident from our experimental and numerical results that the observed increase in MCT1 expression of MCF7-sh-WISP2 cells over the span of a few days is sufficient to ensure survival and sustain proliferation under glucose deprivation, maintaining the population at high cell numbers for about a week, by the end of which we expect cells to have initiated alternative survival mechanisms associated with disease progression *in vivo* [11, 12].

While we recorded an increase in MCT1 expression of glucose-deprived MCF7-sh-WISP2 cells, no MCT4 mRNA was detected, which suggests that the *in vitro* environmental conditions here investigated do not influence the expression of such a monocarboxylate transporter. It would be relevant to perform similar experiments under hypoxic conditions, as hypoxia-regulated signalling pathways may explain the increase in MCT4 expression observed *in vivo* far from tumour blood vessels. In this regard, it would also be significant to formulate a spatially-explicit extension of the present model where oxygen dependency of various dynamics, here ignored as experiments were carried out in normoxic conditions, was modelled explicitly. Such an extended model would allow for theoretical studies on the still debated role of hypoxia in MCT1 expression at tissue level, which might reconcile reported discrepancies between oxygen and pH profiles [4, 45–47], and could inform anti-cancer therapeutic approaches based on MCT1 blockers [13, 14].

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