Cancer Glycolysis I: Entropy Production and Sensitivity Analysis in Stationary State

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Abstract

Using sensitivity analysis and entropy production rate 10 main reactions, among 20 regulating the glycolysis process of AS-30D hepatoma and HeLa tumor cell line in stationary state, were identified. In fact, these 10 fundamental reactions are potential targets in cancer therapy. A high correlation between entropy production rate and intracellular pH_i was detected in this work.

Keywords: Cancer glycolysis; Entropy production rate; Sensitivity analysis

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Introduction

Cancer is a generic name given to a complex interaction network of malignant cells that have lost their specialization and control over normal growth. This network of malignant cells could be considered as a nonlinear dynamical system, self-organized in time and space, far from thermodynamic equilibrium, exhibiting high complexity [1], robustness [2] and adaptability [3].

Nowadays, cancer is the first cause of death in the world according to reports from the WHO (World Health Organization) [4]. There were 14 million of new cases and 8.2 million cancer related deaths in 2012. More than 60% of the new annual cases worldwide appear in Africa, Asia, Central and South America. These regions represent the 70 % of cancer related death in the world. Predictions dictate an increase of the annual cancer cases from 14 million in 2012 to 22 million in the next two decades.

Despite the achievements in molecular biology and genomics, our understanding of tumor cell growth mechanism and the nature of its robustness is not sufficient. In addition, is extremely difficult to achieve an early diagnostic.

Less than 60% of cancers can be cured with current therapies. This is because frequently these therapies produce serious side effects leading to death of the patients instead of killing the disease [5] For these reasons, the search for new, effective and less invasive therapies represents a basic aspect in cancer research.

In the last years cancer glycolysis has been a target in oncology [6]. Most of tumor cells show a high glycolytic rate compared with normal cells. This phenomenon is known in the literature as Warburg's effect and it appears without an increase in the tricarboxylic acid cycle (TAC) or the electron transport chain (ETC) rate [7].

The Warburg's effect is a consequence of an imbalance between maximum glycolysis rate and minimum pyruvate oxidation rate. This high glycolysis rate exceeds the maximum activity of the pyruvate dehydrogenase complex. In order to avoid the accumulation of pyruvate, the cells transform it into lactate through lactate dehydrogenase (LDH). The lactate can be released to the extracellular environment allowing the proliferative cells to take advantage of the high rate of glycolysis [6,7].

This metabolic feature makes the cancer more robust and aggressive, resulting in an increase of the intracellular pH_i and the later acidification of the extracellular environment [8].

In cancerous tissues, low pH values for extracellular microenvironment can produce an increment in secretion and

activation of proteases, and also promotes degradation and restructuration of the extracellular matrix through the activation of proteolytic enzymes. This contributes to invasion and metastases [9,10].

The significant increase of glycolysis rate observed in tumors has been recently verified, yet few oncologists or cancer researchers understand the full scope of Warburg's work [6,7] and its great importance. Without the use of Warburg's seminal discovery, cancer can never be truly cured merely treated (although ineffectively) because when a cancer returns after it has been in remission (which is often the case) treatments fail and the patient has a high probability of death.

We showed in previous studies, the way entropy production per unit time can be used to select the main steps in a complex chemical reactions network, like Belousov-Zhabotinsky reaction [11,12]. Moreover we also showed that entropy production is a specific behavior of tumor, related with cancer robustness and prognostic of the disease.

The identification of the most important reactions involved in the regulation of the glycolytic pathway is a useful strategy to define therapeutic targets in oncology, thus can be a crucial step in cancer drug development. In addition, research on the influence of intracellular pH_i in the robustness of the glycolytic mechanism in neoplastic cells is equally important to understand the cancer biology.

The goal of this work is to extend the thermodynamic formalisms previously developed [13] to the dynamical behavior for glycolysis network model of AS-30D and HeLa cell lines in stationary state. The manuscript is organized as follows: Section 2 is devoted to employed methods. The results are presented in section 3. Finally, some concluding and remarks are presented.

Methods

A stability analysis, sensitivity analysis and numerical simulations

Sensitivity analysis [14] quantitatively investigates the change in response of a system with changes in parameter values. Let us consider a network model consisting of n chemical species involved in m reactions. By the Law of Mass Action, we may formulate the associated kinetic equations, as a system of ordinary differential equations ODE, such as

$$\frac{dC}{dt} = f(C; \lambda) \text{ for all } C \in R^n; \lambda \in R^m; C(0) = C_o \quad (2.1)$$

where λ is the parameter vector, and C is the vector of intermediary concentrations. We may state the problem of sensitivity analysis as that of identifying the alterations caused in the solutions of (2.1) by modifications in the parameter vector λ.

Naturally, when the components of vector λ are the constants associated with the reactions, the above statement is equivalent to investigating how each reaction affects the overall behavior of the dynamical system.

Let us write the solutions of (2.1) as functions of time, initial concentrations and the corresponding parameters:

$$C = C (C_o, \lambda, t) \quad (2.2)$$

Then, sensitivity coefficients may be calculated as:

$$\frac{\partial C(C_o, \lambda, t)}{\partial \lambda} = \Theta(C_o, \lambda, t) \quad (2.3)$$

Where $\Theta(C_o, \lambda, t)$ is an $n \times m$ matrix depending upon initial conditions, time and parameters. The element of this matrix measures the local sensitivity of the system to the change of concentrations.

Let the matrix $\Theta(C_o, \lambda, t)$, such as:

$$\Theta(C_o, \lambda, t) = \begin{bmatrix} \theta_{1,1} \cdots \theta_{1,m} \\ \cdot \\ \cdot \\ \cdot \\ \theta_{n,1} \cdots \theta_{n,m} \end{bmatrix} \quad (2.4)$$

Where $\theta_{i,j} = \dfrac{\partial C}{\partial \lambda_j}$ and $C = (C_1 \cdots C_n)$, $\lambda = (\lambda_1 \cdots \lambda_m)$ Then, the column vectors of the matrix (2.4) describe the importance, for the time evolution of specie, of each reaction.

To obtain these sensitivity coefficients, we may calculate the derivatives of (2.1) with respect to the parameters. Then we obtain:

$$\frac{d\partial C_i}{dt\partial \lambda_j} = \frac{\partial f_i}{\partial \lambda_j} + \sum_k \Im_{i,k} \frac{\partial C_k}{\partial \lambda_j} \quad (2.5)$$

Where $\Im$ is the Jacobian matrix of the right side equation (2.1).

That is $\Im_{i,k} = \dfrac{\partial f_i}{\partial \lambda_i}$, and $f = (f_1 \cdots f_n)$

There are two basic methods for the solution of (2.5): using a local approach, as the one describe above, or globally, by using Green's Functions. In the former case we may obtain an explicit solution of equation (2.5) from the following linear system:

$$\frac{dG(t, t_0, C)}{dt} = \Im \big[C(t) \big] G(t, t_0, C) \quad (2.6)$$

With the initial conditions:

$$G(t_0, t_0, C) = 1$$

Where $\Im \big[C(t) \big]$ is the Jacobian matrix of (2.1), evaluated through the trajectory C (t). The elements of matrix G are the desired sensitivity coefficients.

Thermodynamics framework

The entropy production per time unit $\dot{S}_i \equiv \dfrac{\delta S_i}{dt}$ with *T,p* fixed and disregarding diffusive and viscous effects, of each reaction of the glycolytic pathway **(Table 1),** was assessed without lost of

generality [15] as

$$\dot{S}_i = -\frac{1}{T}\Delta G_k\,\dot{\xi}_k \qquad (2.8)$$

Where $\dot{\xi}_k$ represent the generalized flux, the reaction rate $\frac{d\xi}{dt} = \dot{\xi}_k$, and $\frac{1}{T}\Delta G_k$ the generalized force, with other words, the variation of Gibbs free energy of the *K-th* reaction of glycolysis pathway.

The Gibbs free energy, of the *K-th* reaction is written [16] as

$$\Delta G_k = \Delta G_k^{\oplus}(T, pH, I) + RT\sum_i \nu_i \ln c_i \qquad (2.9)$$

where: ν_i, c_i represent the stoichiometric coefficients and the concentrations respectively, of the involved biomolecules in each reaction and $\Delta G_k^{\oplus}(T, pH, I)$ is the standard Gibbs free energy adjusted taking in to account its dependence of temperature, *pH* and ionic force $_I$ [17,18], in the physiological conditions used experimentally [19]: T = 310, 15 K, I = 0,18M and *pH* = 7.

The main idea, according to previous works [20], is the following: The reactions which display a high entropy production value per unit of time, are considered in the process. This statement could be considered as extension of the "Principle of Maximum Entropy" [21].

Recently, we extend this formalism to determine the main reactions of glycolysis network model of tumor cells proposed by Marin et al. [19] for HeLa cell line. Our purpose now is to generalize it in order to apply it in hepatoma AS-30D at high concentration levels of Glucose, 5 mM.

The entropy production rate was normalized in percent using as a baseline the highest value due to there is no physical criteria that allow setting a minimum value. The 10 percent was used as an empirical criterion for the minimum value like is shown in the **Table 1**.

Model

A metabolic model of the glycolytic network for HeLa tumor cell-lines under Normoxia (95% O_2) and Hypoxia (0.1-0.5% O_2) conditions, and for AS30D cells (rat hepatoma) proposed by Marin et al. [19] from experimental studies was used.

A stability analysis [22] sensitivity analysis [14] and numerical simulations of glycolysis network model of tumor cells proposed by Marin et al. [19] for HeLa and AS-30D cell lines **(Table 1)**, was carried out by COPASI v. 4.6.32 software available in the website http://www.copasi.org. The parameters and concentration values reported by Marin et al. [19] were used.

Results

The ATP concentration **(Figure 1)** indicates that the stationary state (ss) is asymptotically stable for both cell types, assuming the initial concentration of glucose extracellular (Glu$_{out}$) as a control parameter.

ATP concentration in HeLa cells in ss under hypoxia (7.7 mM) is similar to the concentration of hepatoma AS-30D (7.9 mM) under high glucose levels (Glu$_{out}$= 5 mM). The transient time is also similar for both, ~130 min. Furthermore, AS-30D under low glucose levels (1 mM) display a similar transient time to HeLa cells transient time under normoxia conditions, ~200 min.

The transient times are often called lifetimes as they can also be interpreted as an indication of the lifetime of a single molecule of the metabolite. One can safely refer to metabolites with high transition times as slow and those with small transition times as fast **(Figure 1)**.

Among the reactions of the glycolytic pathway **(Table 1),** using sensibility analysis, five were identified to regulate tumor in the case of HeLa cells **(Figure 2)**. Among these, the reactions 1, 4, and 13 were also identified by Marin et al. [19] using Metabolic Control Analysis, MCA. On the other hand, five reactions were also identified in the hepatoma AS-30D scenery **(Figure 2)**. Three of them, 1, 2, 3, agree with the reactions identified by Marin et al. [19] applying MCA **(Figure 2).**

It is remarkable that the reaction 14 was detected as the most important step in cancer glycolysis regulation in both scenarios. It is known that ATPase (#14) plays a fundamental role in the control of intracellular and extracellular *pH* [9]. In fact, ATPase is up-regulated in many metastatic tumors where a positive correlation between the expression levels and the risks of invasion and metastases processes were found. This overexpression of ATPase gives several advantages to cancer cells over normal cells in the tumor macro environment [23].

The V-ATPase, is a cell-specific proton pump that can be expressed in the plasmatic membranes of human tumor cells and it can have a specialized function in cellular growth, differentiation, angiogenesis, metastases and plays an important role in both, extracellular and intracellular *pH* control [9].

It's involved in keeping a slightly basic (near neutral) $pH_{intracellular}$ (pH_i) and an acid $pH_{extracellular}$ (pH_e), through H^+ pumping from intracellular environment to vacuoles and extracellular environment. This is known as "reversed" *pH* gradient. The V-ATPase is overexpressed in many types of cancer and is related to their capacity of invasion and metastases [10]. V-ATPase is also related to obtaining a cellular phenotype resistant to drugs, so it has been pointed as a potential target for cancer treatment [9].

As can be noticed in **Table 1**, the entropy production per unit time $\dot{S}_i$ of the glycolysis pathway in HeLa cell line in hypoxic conditions is higher than in normoxia. This indicates, not only how is the glycolysis process favored under low oxygen conditions (matching the glycolytic flux values reported by Marin et al. [19], but also that under these conditions it becomes more robust **(Table 1)**.

The $\dot{S}_i$ increases in hypoxia condition can happen due an overexpresion of transcription regulatory mechanisms that favour low oxygen glycolytic route as HIF-1 (Hypoxia Induced Factor 1).

It is known that tumors in hypoxia conditions are more resistant and aggressive [24]. Also, it can be observed, that for AS-30D, despite the glycolytic flux is identical to HeLa, in hypoxia conditions, the entropy production rate is slightly superior of that of AS-30D hepatoma.
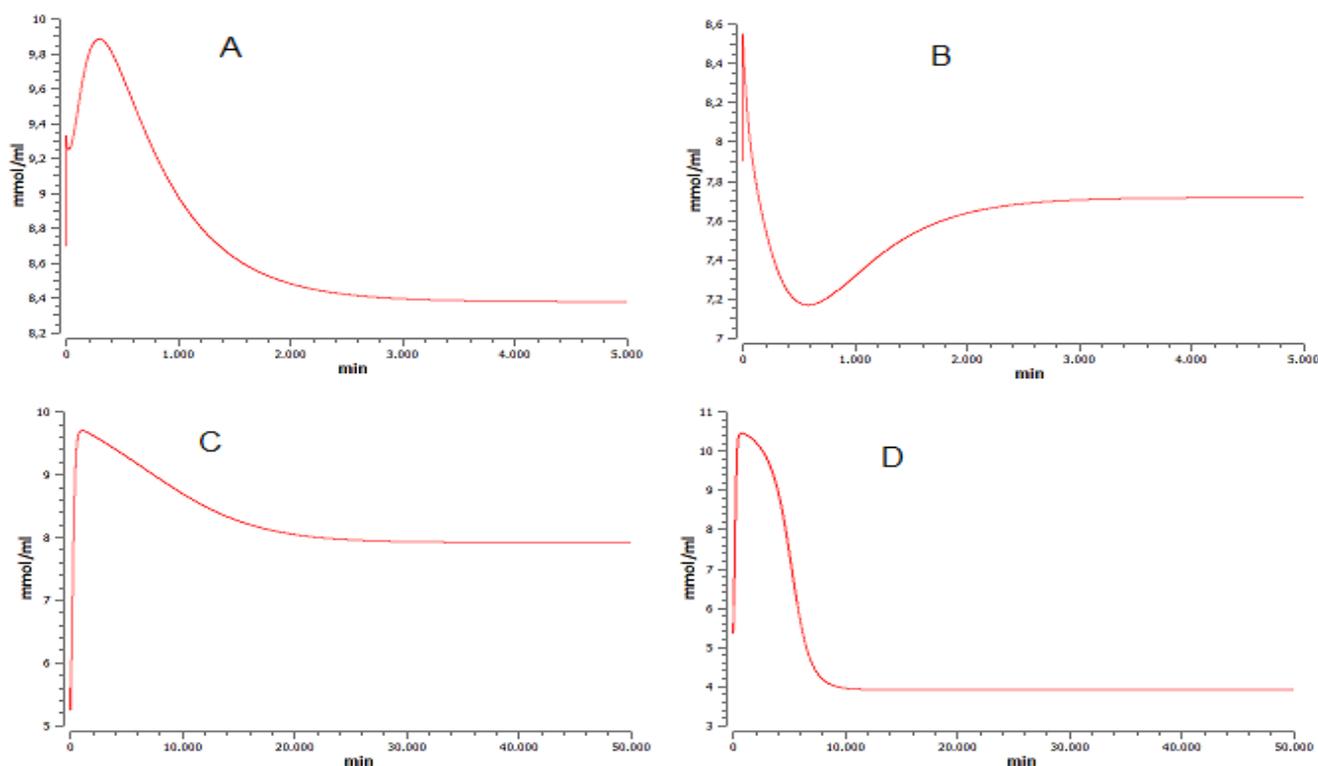
Figure 1 Dynamical behavior (**ATP** concentration) of the glycolysis network model of HeLa (**A**,**B**) and AS-30D (**C,D**) tumor cells: **A** ($[Glu_{out}]_0$= 5 mM, normoxia), **B** (($[Glu_{out}]_0$= 5 mM, hypoxia), **C** (($[Glu_{out}]_0$= 5 mM,)**, D** (($[Glu_{out}]_0$= 1 mM).
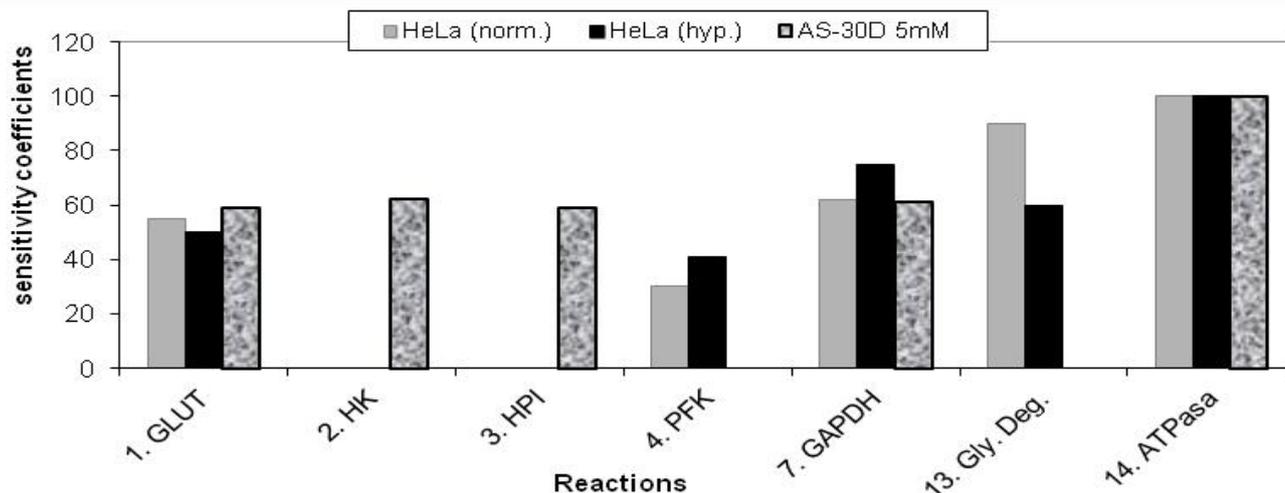

Figure 2 Normalized values of sensitivity coefficients in key reactions identified according to the glycolysis network model of tumor cells lines.

As shown in **Table 1**, 9 of 20 reactions of the glycolysis network model were identified. For HeLa cells, 3 matches the ones found previously by sensitivity analysis (4,7,14) and 3 with the ones found [19] by MCA (2,3,4). In hepatoma, 4 matches with sensitivity analysis results (2,3,7,14) and 3 with [19] MCA (2,3,4).

GAPDH (reaction 7) was identified as the second most important reaction for both cell lines. Evidence suggests that GAPDH plays a role in apoptosis when this is translocated to nucleus, although its mechanism and function it's still to be described, this suggest that GAPDH is a possible link between glycolysis and apoptosis [25].

ENO (reaction 10), is also identified as fundamental. Importance of this enzyme for cancer lies in the fact that is capable of acting as plasminogen6 receiver, enhancing growth and dissemination of tumoral cells [26].

Table 1 Entropy production rate and normalized values (%) for glycolysis network model of AS-30D and HeLa tumor cells [19].

Reaction	Enzyme or branch	HeLa normoxia $\left[\dfrac{\dot{S}_i}{mM \cdot K \cdot min}\right]$	(%)	Hypoxia $\left[\dfrac{\dot{S}_i}{mM \cdot K \cdot min}\right]$	(%)	AS-30D 5 mM Glucose $\left[\dfrac{\dot{S}_i}{mM \cdot K \cdot min}\right]$	(%)
1. Gluout = Gluin	GLUT	♣0,088*	3	♣0,102*	3	♣0,053*	1
2. Gluin + ATP = G6P + ADP	HK	**0,463***	16	**0,939***	23	♣**1,385***	32
3. G6P = F6P;Ery4P, FBP, 6PG	HPI	0,247*	8	0,334	8	♣0,546*	13
4. F6P = FBP;ATP	PFK1	♣**0,680***	23	♣**0,932**	23	0,678*	16
5. FBP = DHAP + G3P	ALDO	-0,692	-	-1,017	-	-1,001	-
6. DHAP = G3P	TPI	**0,527**	18	**0,782**	19	0,863	20
7. NAD + G3P + Pi = 1,3BPG + NADH	GAPDH	♣**1,822**	61	♣**2,751**	67	♣**2,313**	54
8. 1,3BPG + ADP = 3PG + ATP	PGK	0,039	1	0,058	1	**0,520**	12
9. 3PG = 2PG	PGAM	-0,347	-	-0,515	-	-1,001	-
10. 2PG = PEP	ENO	**0,777**	26	**1,155**	28	**1,161**	27
11. PEP + ADP = Pyr + ATP;FBP	PYK	0,075	3	0,213	5	**1,058**	25
12. NADH + Pyr = Lac + NAD	LDH	0,188	6	0,285	7	0,058	1
13. glycogen + Pi → G6P	GLYC DEG	♣0,006*	0,2	♣0,006*	0,2	≈0	0
14. ATP → ADP + Pi	ATPasa	♣**2,974**	100	♣**4,101**	100	♣**4,307**	100
15. ATP + AMP = 2*ADP	AK	≈0	0	≈0	0	≈0	0
16. NADH = NAD	DHases	0,017	0,6	0,017	0,4	0,084	2
17. G6P → 6PG	PPP	≈0	≈0	≈0	0	≈0	0
18. G6P + ATP → glycogen + ADP + 2*Pi	GLYC SINT	≈0	0	≈0	0	≈0	0
19. Pyr + 13*ADP + 13*Pi → 13*ATP	MPM	≈0	≈0	≈0	0	≈0	0
20. Xy5P + Ery4P → G3P + F6P	TK	≈0	≈0	≈0	0	≈0	0
TOTAL		6,866		10,144		11,028	
Glycolytic flux [19]		**20**		**29**		**29**	

Hexokinase (HK, reaction 2) is identified as a main one in the glycolytic route. Its isoform HKII was reported by Mathupala et al. [27] as the "Cancer Double-Edge Sword". It eases and protects the tumor malignancy when it's bonded to mitochondria. On the other hand, its mitochondrial activity shows that HK is required by grow factors inductors of the cellular survivor Also the joint between HK and the mitochondrial membrane rules apoptosis inhibition, still, it mechanism remains unknown. HK is important for keeping the high speed of glycolysis and for tumour survival [28].

In normal differentiated adult cells, intracellular pH is generally ~7.2 and lower than the extracellular pH of ~7.4. Deregulated pH is emerging as a hallmark of cancer [29] because cancers show a 'reversed' pH gradient with a constitutively increased intracellular pH_i that is higher than the extracellular pH_e.

An increased intracellular pH_i is permissive for cell proliferation and the evasion of apoptosis, facilitates metabolic adaptation and is obligatory for efficient directed cell migration, For this purpose, entropy production rate was evaluated for different values of pH_i ranging from 6.2 to 7.4. In **Figure 3** an excellent correlation is shown between entropy production rate $\dot{S}_i$ for reaction #14 (ATPase), as a function of intracellular, pH_i for glycolysis network model for AS-30D and HeLa cells **(Figure 3)**.

As observed, when intracellular pHi gets lower, also does the entropy production rate, which measures the loss of the process robustness with lower intracellular pH_i. The inverted pH gradient between the inside and the outside of cells that is observed in tumors presents both challenges and opportunities for drug discovery [29]. Indications are that acidification of intracellular environment is cytotoxic to tumor cells [30-36].

These results demonstrate that, the pH_i regulatory mechanism may be an effective therapy target, because inhibition of pH_i regulation will cause a decrease of pH_i preferentially in tumor cells in acidic extracellular environment relative to normal cells and thus cause damage preferentially in tumor cells.
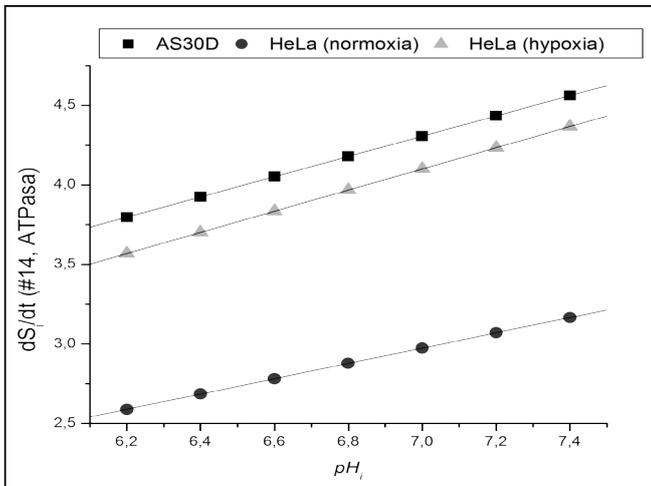
Figure 3 Entropy production rate $\dot{S}_i$ for reaction #14 (ATPase) as a function of intracellular pH_i, for glycolysis network model for AS-30D and HeLa cells.

This way, we can see that reaction 14, is a potential target of the glycolysis process in cancer treatment, not only because is the most important one in the regulation of the glycolytic pathway, but also because is the one with the highest dependence with intracellular pH_i [37-40].

Conclusions and Remarks

The results show how the thermodynamic approach constitutes an effective tool combined with the application of sensitivity analysis to study the cancer glycolysis process. As a matter of fact, we found more evidences that manifest that the entropy production rate represent a physical magnitude that measures the cancer robustness.

In this paper we found

- Using sensitivity analysis and the entropy production rate that 10 reactions, among the 20 of the glycolysis network model, are fundamental. These reactions constitute potential targets in cancer therapy.

- In cancer glycolysis under hypoxia conditions, the entropy production rate is higher than the entropy production rate of normoxia that means more complexity and robustness. This conduces to the thesis that the employ of any type of therapy has to be in normoxia conditions, as a key factor in the way to improve the cancer therapies.

- We showed the existence of an excellent correlation between the entropy production rate and the intracellular pH.

In summary, the regulation of glycolysis, relevant to senescence process, would be a key to improve and identify new anti-cancer therapies in the future. The current theoretical framework will hopefully provide a better understanding of cancer and contribute to improvements in cancer treatment.

Acknowledgements

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