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Physica A **436**, 246-255, 2015

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Abstract

Cancer can be considered as an open, complex, (bio-thermo)dynamic and self-organizing system. Consequently, an entropy generation approach has been employed to analyze its mitosis/apoptosis ratio. Specifically, a novel thermodynamic anticancer strategy is suggested, based on the variation of entropy generation caused by the application of external fields, for example electro-magnetic fields, for therapeutic purposes. Eventually, this innovative approach could support conventional therapies, particularly for inoperable tumors or advanced stages of cancer, when larger tumor burden is diagnosed, and therapeutic options are often limited.

Keywords: Apoptosis, mitosis, entropy, irreversibility, apoptosis/mitosis ratio.

INTRODUCTION

Recently, a new interdisciplinary approach [1], based on a thermodynamic analysis of the irreversibility of the open real systems, has been introduced to analyse the V-ATPase mechanism and its consequences in the behaviour of the cell. An improvement of this approach consists in the analysis of the mitosis/apoptosis processes, which is the object of this paper.

Conventionally, cancer is understood as a set of malignant cells, which lost their growth control and which exhibit eventually an invasive and metastatic phenotype through a process called carcinogenesis [2-6]. As a consequence, their number and density increases, and they spread. But, recently, other related properties of cancer systems have been highlighted, i.e.:

1. Precursor cells, often present in cancers, emphasize a relationship between cancer cells and their stroma. In fact, a fundamental interaction between the tumor and its environment has been emphasized [7-11].
2. Neovascular blood vessel formations that nourish cancer growth [3], (regardless of the fact that avascular tumor growth conditions have been discovered also [4]).

Moreover, some other results must be considered [4]:

i. There are genes that control mitosis and apoptosis, and that are differentially expressed in cancer cells; these genes are expressed also in pre-neoplastic states;
ii. Some genes related to the cancer's growth potential and to its invasive behaviour;
iii. Cancer has an advantageous mitosis-to-apoptosis turnover ratio in that many more cells are generated through replication while comparatively less cells die [4-6].
iv. Biopsy samples obtained from macroscopically normal organs may contain foci of partially transformed tissue, quiescent only until they are in contact with fibroblasts, which send out physiological growth signals [3].

As such, cancer emerges through a series of steps thought to be sequential, as a disease of abnormal growth driven by local cellular expansion, adjacent tissue infiltration, and distant metastases. Consequently, one of the fundamental approaches to carcinogenesis consists of investigating the derangement of mitosis and, perhaps more so, of the mitosis/apoptosis ratio, which will lead to such an abnormal large mass [12].

In normal cells, processes such as DNA replication, DNA transcription and RNA translation convert molecular binding energy, chemical bond hydrolysis and electric gradients into mechanical work, related to conformational changes and displacements [13]. Still, the origin of this mechanical conversion of energy is not yet completely understood; as such, a better insight into the signaling pathways that process cell proliferation and also into the mitosis/apoptosis ratio could lead to new therapeutic approaches to diseases, particularly with regards to cancer [11].

Recently, an entropy generation concept based on the Gouy-Stodola theorem [14] has been suggested as a powerful approach to analyze not only the cells' biochemical and biophysical processes [8-10], but also their statistical and chemo-thermodynamic pathways [11,15].

The aim of this paper is to introduce this approach to the mitosis/apoptosis ratio in an effort to obtain a new method of analysis for these processes, and to suggest implementing an external field as novel treatment modality in support of currently applied anticancer regimen. To do so, in Section 2, a summary of the thermodynamic approach is presented, while, in Section 3, it will be discussed from a biomedical point of view. This is followed by Section 4, which presents relevant *in vitro*, *in vivo* and *clinical* data from the literature prior to concluding remarks summarizing our findings, both theoretically and experimentally.

THE THERMODYNAMIC APPROACH

In this Section, we summarize some recent thermodynamic results that are useful to develop the biomedical arguments.

In applied thermodynamics, the quantitative description of irreversibility is obtained by

introducing the concept of entropy generation. Cells are open and complex systems, and, as such, they can be analysed by using an applied thermodynamics approach. Consequently, it can be useful to understand the thermodynamic bases of self-organization within the realm of the evolution of order and life [8]. First, the entropy generation of any open system is defined as [16]:

$$S_g = \int_0^{\tau} \left[\frac{dS}{d\tau} - \sum_{i=1}^{n} \frac{\dot{Q}_i}{T_i} - \sum_{in} G_{in} s_{in} + \sum_{out} G_{out} s_{out} \right] d\tau \tag{1}$$

where τ is the lifetime of the process, which can be defined as the range of time in which the process occurs [9,14,15]; Q stands for the heat exchanged, T is the temperature of the thermal source, s represents the specific entropy and G is the mass flow. In relation to cells, the entropy generation has recently been evaluated as [8]:

$$\begin{aligned} S_g &= -\int_o^{\tau_1} \frac{\nu}{T^2} \mathbf{J}_q \cdot \nabla T dt - \int_o^{\tau_2} \nu \sum_k \mathbf{J}_k \cdot \nabla \left(\frac{\mu_k}{T} \right) dt - \int_0^{\tau_3} \frac{\nu}{T} \mathbf{\Pi} : \nabla \dot{\mathbf{x}}_B dt - \int_0^{\tau_4} \frac{\nu}{T} \sum_j J_j \mathcal{A}_j + \int_0^{\tau_5} \frac{\nu}{T} \sum_k \mathbf{J}_k \cdot \mathbf{F}_k \\ &= S_{g,tf} + S_{g,dc} + S_{g,vg} + S_{g,cr} + S_{g,de} \end{aligned} \tag{2}$$

where:

1. $S_{g,tf}$ is the entropy generation due to the thermal flux driven by temperature difference, which was obtained as $S_{g,tf} \approx \frac{uL^2 \dot{x}_{th}}{6T^2} \Delta T \tau_1$
2. $S_{g,dc}$ is the entropy generation due to the diffusion current driven by chemical potential gradients, which was obtained as $S_{g,dc} \approx \frac{\dot{x}_{th} V_m}{T} \frac{\sum_i \rho_i \left(\mu_{i,os} - \mu_{i,is} \right)}{d_m} \tau_2$
3. $S_{g,vg}$ is the entropy generation due to the velocity gradient coupled with viscous stress, which was obtained as $S_{g,vg} \approx \frac{4\pi}{T} \eta \frac{\dot{\mathbf{x}}_B^2}{r d_e} \tau_3$
4. $S_{g,cr}$ is the entropy generation due to the chemical reaction rate driven by affinity, $S_{g,cr} \approx V \tau_4 \sum_i N_i \frac{\mathcal{A}_i}{T}$

5. $S_{g,de}$ is the entropy generation due to the dissipation generated by interaction with the environment which was obtained as $S_{g,de} \approx -\int_V dV \int_0^{\tau_5} \frac{\nu}{T} \sum_k \mathbf{J}_k \cdot \mathbf{F}_k$

where τ_i, $i \in [1,5]$, is the lifetime of any process, and L is a typical length of a cell (which can be evaluated as its diameter if it is approximated as a sphere) and ΔT is the temperature difference between the cell and its environment; μ_i are chemical potentials of the i-th species, V_m and d_m are the volume and depth of the membrane, where the chemical potential gradient, $\Sigma_i \rho_i (\mu_{i,os} - \mu_{i,is})/d_m$, occurs especially in cytoplasm, $\dot{\mathbf{x}}_{th}$ is the thermal velocity, ρ_i is the concentration of the i-th species and *os* and *is* means *outside* and *inside* the cell, respectively, while T represents the mean temperature of the membrane; η stands for the average viscosity coefficient, $\dot{\mathbf{x}}_B^2$ denotes the center of mass velocity of all components in a cell, d_e the cytoplasm layer and r the mean cell radius; lastly, N is the number per unit time and volume of the i-th chemical reaction and $\mathcal{A}$ is the affinity, **F** is the force generated by the interaction with the external field and **J** stands for the associated flow. Moreover, the exergy of a system is defined as the maximum shaft work that could be done by the composite of the system and a specified reference environment that is assumed to be infinite, in equilibrium, and ultimately to enclose all other systems: the environment is specified by stating its temperature, pressure and chemical composition.

Starting from these results a relation between the temperature difference between the cell and its environment and the cell diameter has been obtained as follows [9]:

$$\Delta T = \frac{L_0^2}{L^2}\Delta T_0 + \frac{2\gamma}{3\alpha}\left(\frac{1}{L_0^2 L^2} - \frac{1}{L^4}\right) - \frac{\varepsilon}{\alpha}\left(\frac{1}{L} - \frac{L_0}{L^2}\right) - \frac{\beta - \kappa}{\alpha}\left(L - \frac{L_0^3}{L^2}\right) \tag{3}$$

with L_0 the diameter of the daughter cell at its birth and ΔT_0 the temperature difference between the cell and its microenvironment at the outset; the constants in equation (3) are defined as follows [1,11]:

1. $\alpha = \frac{3.3 \times 10^{12} \tau_1}{T}$, with τ_1 in the range 15÷269 ms

2. $\beta = \frac{3\times10^{7}\tau_2}{T}$, with $\tau_2 \approx 10$ s

3. $\gamma = \frac{4.7\times10^{12}\tau_3}{T}$, with $\tau_3 = \frac{2\pi r}{c}$, with $c \sim 1540$ m s^{-1}

4. $\varepsilon = 0.523\,\tau_4 \sum_i N_i \frac{\mathcal{A}_i}{T}$, with τ_4 in the range of 17÷1283 ns

5. $\kappa = \frac{\pi}{6}\frac{v\tau_5}{T}\sum_k \mathbf{J}_k \cdot \mathbf{F}_k$, with τ_5 dependent on the interaction considered.

These values are obtained by using some numerical approximations and data from the literature [1,8-11,17-29]. Furthermore, the volume of a cell is approximated by a mean cell sphere with the diameter,

$$L = \left(\frac{6V}{\pi}\right)^{1/3} \tag{4}$$

with V being the cell volume. The mean cell temperature can be assumed as T = 310 K; ΔT = 0.4°C, but this quantity would be experimentally evaluated for different cells lines and it is different between normal and cancerous (or more generally, diseased) states. The characteristic length can be evaluated as $L = 2\,r$; the internal energy density can be evaluated as the ratio between the cell mean internal energy, considered the same as that of [1,6] ATP, $U = 3\times10^{-7}$ J, and the mean value of the cell assumed to be [1,6] $V = 7600$ μm^3, with the cell volume in the human body being in the range [6,29,30] 200-15000 μm^3, which leads to $u = 3.95\times10^{7}$ Jm^{-3}; the thermal molecular mean velocity inside the cytoplasm is considered to be [1,6] $\dot{x}_{th} = 5\times10^{-5}$ m s^{-1} and the membrane volume is evaluated as

$$V_m = \frac{4}{3}\pi r^3 - \frac{4}{3}\pi(r-d_e)^3 = \frac{4}{3}\pi r^3 - \frac{4}{3}\pi(r-0.2r)^3 = \frac{4}{3}0.992\pi r^3 = 0.992V \tag{5}$$

We note that the chemical potential gradient can be evaluated as the ratio between the mean value of the chemical potential [1,6] $\mu = 1.20\times10^{-9}$ J kg^{-1} and the membrane length [1,29] $d_m = 0.01$ μm, being the mean density $\rho = 1000$ kg m^{-3}; the viscosity is evaluated as [1,6] 6.91×10^{-3} N s m^{-2}; d_e can be evaluated as $d_e = 0.2\,r$; $\eta \sim 2.07\times10^{-3}$ N s m^{-2} [1,29] at 30°C [1,6]; $\dot{x}_B$ is evaluated as 3.0×10^{-6}

m s^{-1} [1,29].

The external fields are theoretically weighted by the constant κ. Their contributions depend on the kind of fields considered, for example an electro-magnetic wave. Considering relation (3) we can argue that if there are no external fields the coefficient $(\beta - \kappa)/\alpha$ becomes β/α, while if there are external fields it remains $(\beta - \kappa)/\alpha$. This variation in the coefficient determines a variation in the temperature difference $\Delta T - \Delta T_0$; consequently, the growth behaviour of the cell changes [8-11].

Moreover, in cells many processes involve biological macromolecules. One of their fundamental properties is their allosteric transition: when a process occurs at one border, it can lead to a change in the configuration of another site of the same macromolecule, and it can change inside the same tissues. This is familiar from the regulation of enzyme activity, motor proteins, ion transport through membranes, and others. When the temperature increases, the amplitude of macromolecule oscillations will increase with a consequent decrease in the enzyme's activity. Such a thermalization of this oscillator-like process takes a characteristic time, which depends on the interaction between the cell and its environment. It is possible to argue that the external field could modify this thermalization process. The combined effect of the membrane interaction with external fields and the thermalization interaction with macromolecules can represent a possible thermodynamic approach to cell behaviour in order to obtain some explanation of disease states [1,8-11].

AFFINITY AND APOPTOSIS/MITOSIS RATIO

In this Section, the aforementioned approach will be used to develop a thermodynamic analysis of the mitosis/apoptosis ratio in tumors.

To do so, it is necessary to introduce some results obtained through the analysis of cancer geometry. Cells have a fractal geometry [3,31-34]; consequently, cell mass increases as a function of a characteristic length, which can be the diameter. So, it is possible to write:

$$M = \zeta V^{d_f/3} = \left(\frac{\pi}{6}\right)^{d_f/3} \zeta\, L^{d_f} = \zeta'\, L^{d_f} \tag{6}$$

with [3] $2 < d_f < 3$ being the fractal dimension for a three dimensional approach, and ζ and ζ' being proportional constants. But, the fractal dimension was proven to be related to the mitosis/apoptosis ratio [4]:

$$d_f = d_f\left(\frac{\chi_1}{\chi_2}\right) = d_f\left(\frac{P_m}{P_a}(1+F_a)\right) \tag{7}$$

with χ_1 representing a cell reproduction rate constant and χ_2 the cell death rate constant, defined as:

$$\begin{aligned} P_m &= \chi_1\, n^{1/d} \\ P_a &= \chi_2\,(1+F_a)\, n^{1/d} \end{aligned} \tag{8}$$

where n is the number of cells, P is the probability per unit time, m means mitosis, a stands for apoptosis, F_a is a dimensionless correction term which represents the relation between the cancer mass radius and a characteristic length of the volume, and it takes into account the finite size of the host organ or tissue, and d is a constant.

From these relations the entropy generation due to affinity was obtained as a function of χ_1 and χ_2, and using relation (8) the mitosis and apoptosis probability was obtained as [11]:

$$S_{g,cr} \approx k\left(P_m - \frac{P_a}{1-F_a}\right)\left[\ln\left(\frac{P_m}{P_a}\right) + \ln(1+F_a)\right] = K\tau_4\left(\dot{\xi}_f - \dot{\xi}_b\right)\ln\left(\frac{\dot{\xi}_f}{\dot{\xi}_b}\right) \tag{9}$$

where $\dot{\xi}_f$ is the forward reaction rate and $\dot{\xi}_b$ the backward reaction rate [35]. It then follows that:

$$\begin{aligned} P_m &= \frac{Kn^{1/d}}{k}\tau_4\dot{\xi}_f \\ P_a &= \frac{Kn^{1/d}}{k}\tau_4(1+F_a)\,\dot{\xi}_b \end{aligned} \tag{10}$$

This equation highlights the direct relation between the probability and the time of the reaction, while the ratio between the two probabilities highlights the fundamental role of the

chemical reaction rate in the dynamics of tumor growth, but also the critical role of the geometric factor ($1 + F_a$). From relations (3) and (6) we can obtain the cancer mass. Furthermore, it is possible to obtain the variation of this mass as a function of the variation of the temperature difference; it results:

$$\frac{dM}{d\Delta T} = \frac{\alpha \zeta' d_f \, L^{d_f+4}}{2\gamma - \varepsilon L^3 - 2\alpha L^4 \Delta T - 3(\beta - \kappa)L^5} \tag{11}$$

from which the mass of the cancer tissue can be obtained as:

$$\Delta M = \frac{\zeta' d_f \, L^{d_f}}{2} \ln\left(\frac{2\gamma - \varepsilon L^3 - 2\alpha L^4 \Delta T - 3(\beta - \kappa)L^5}{2\gamma - \varepsilon L^3 - 2\alpha L^4 \Delta T_0 - 3(\beta - \kappa)L^5} \right) \Rightarrow$$

$$\Delta M = \frac{\zeta' d_f \, L^{d_f}}{2} \ln\left[\frac{2\gamma - \varepsilon L^3 - 2\alpha L^4 \left[\frac{L_0^2}{L^2} \Delta T_0 + \frac{2\gamma}{3\alpha}\left(\frac{1}{L_0^2 L^2} - \frac{1}{L^4} \right) - \frac{\varepsilon}{\alpha}\left(\frac{1}{L} - \frac{L_0}{L^2} \right) - \frac{\beta - \kappa}{\alpha}\left(L - \frac{L_0^3}{L^2} \right) \right] - 3(\beta - \kappa)L^5}{2\gamma - \varepsilon L^3 - 2\alpha L^4 \Delta T_0 - 3(\beta - \kappa)L^5} \right] \tag{12}$$

This relation highlights that the effect of external fields can be obtained only if

$$\kappa \geq \beta \tag{13}$$

because under this condition the sign of the coefficient β - κ can change and as a consequence the tumor mass growth changes its behavior also. Consequently, considering that $\beta \sim 10^6$ J m^{-3} K^{-1}, then κ results in the same order of magnitude. Now, considering an ion current of $J_k = I/A$, being A the mean surface of the membrane, of the order of 10^{-4} A, $\tau_5 \sim 10^{-14}$ s, $\nu \sim 10^{-3}$ m^3 kg^{-1}, T = 310 K, it follows that the electric field $F_k = E/V$, with V, mean volume of the cell, in the order of 10^{-15} m^3, must be of the order of $10^7 \div 10^9$ V m^{-1} and the relative magnetic field $B = \sqrt{\mu_m \varepsilon_e} E$ results around $10^{-5} \div 10^{-4}$ T. The frequency can be evaluated considering that the ionic current I across the membrane can be obtained as:

$$I = \frac{N}{t} e \tag{14}$$

where e is the electric charge (~10^{-19} A s) and N is the number of ions which cross the membrane (~ 10^8 particles s^{-1}). This leads to $t \sim 1 \div 10^{-3}$ s, and consequently, the frequency of the magnetic wave would be $1 \div 10^3$ Hz.

Equation (12) is evaluated in Figure 1 for cancer growth outside an external field and in Figure 2 for a cancer growth inside an external field, such as a magnetic field, which we can conjecture being in the order of the Earth's magnetic field (~ 40 μT) with a frequency of ~ 50 Hz. The two shapes are qualitative because we considered a mean value of diameter for a mean cell (1.97×10^{-5} m). We note that the external field (~ $10^7 \div 10^9$ V m^{-1}) can be an electric or a magnetic field (~$10 \div 10^2$ μT). The resultant shape of Figure 1 is in agreement with the S-shape for a tumor growing according to the Gompertz law, highlighting the goodness of the thermodynamic model. Figure 2 depicts how an external field inhibits cancer growth as the maximum mass of the tumor is considerably reduced (versus Figure 1). In Figure 3, however, we present a more clinically realistic situation: a patient is subjected to this novel anticancer therapy after the cancer is diagnosed, i.e. not at time 0 as in Figure 2. The shape in Figure 3(a) is related to a relative growth of 0.4; the inhibitory effect on tumor mass and delay in growth is evident. In these runs here, the external field leads to much improved tumor control, rather than cure; cautiously extrapolated, it is therefore ideally applied in support of or to amplify more conventional, adjuvant anticancer regimen (such as radiotherapy and/or chemotherapy) or in situations with inoperable tumors, for instance. The next Section supports this notion of field therapy having anti-cancer efficacy by citing relevant experimental *in vitro* and *in vivo* data, as well as some clinical works from the literature, which then leads us to conclude with a comparison of our own experimental *in vitro* and *in silico* modeling data in the final Section 5.

EXPERIMENTAL RESULTS

Indeed, a large number of studies have been carried out to investigate the effects of electromagnetic fields in biological systems [36-49]. Relevant *in vitro* studies can be summarized as:

1. Human cervical cancer and rat pheochromocytoma cells show a 18.4% and 12.9% decrease, respectively, in cell proliferation when exposed continuously for 72h to a magnetic field of 1.2 ± 0.1 mT, at 60 Hz [41];
2. Human cervical cancer cell proliferation decreased by 15% 24h after being exposed to a magnetic field of 0.18T, at 0.8Hz, for 16h [42];
3. Rat pheochromocytoma cells exposed to a 50 Hz magnetic field showed also morphological differentiation [43];
4. Human colon adenocarcinoma cells decreased their growth when exposed to 1Hz for 6 h [44];
5. Finally, the cell number of melanoma cells declined by 19.04 ± 7.32 %, that of ovarian carcinoma by 22.06 ± 6.19 %, and that of lymphoma by 40.68 ± 8.31 % when exposed to a 7 T uniform static magnetic field [45].

Moreover, there are many othermore than sufficient experimental *in vivo* data supporting our results of anti-cancer and more specifically anti-proliferative effects of electromagnetic waves in solid tumor types [38-70]. Such *in vivo* studies were routinely conducted in rodent models. Overall, these studies were concordant on the inhibition of cancer cell growth, albeit it seems difficult to compare them due to the enormous variation in cell types and fields employed. Strikingly, electromagnetic fields seemed to be most effective in their inhibition of cancer cell growth at moderate and at low intensity. Novikov et al. exposed a total of 1750 BALB/c mice to alternating fields at frequencies ranging from 0.5 to 16.5 Hz and intensities from 100 to 300 nT [67]. Mice were intraperitoneally injected with 1×106 Ehrlich ascites cancer cells, which caused zero survival of control group animals within 13-18 days. On the contrary, 82% and 60% of electromagnetic fields exposed mice were alive at 25 days after treatment with a sum of frequencies (1 Hz, 4.4 Hz

and 16.5 Hz) or at 4.4 Hz 100 nT, respectively; noteworthy, this study was performed using parallel static and alternating magnetic fields [67]. Other relevant studies include:

1. Using the nude mouse animal model, A-Mel-3 melanomas were exposed for 3 h to magnetic fields of less than 600 mT: a deceleration of tumor growth was observed whereas angiogenesis was attenuated [47]; a magnetic field of about 150 mT resulted in a significant reduction of red blood cell velocity and segmental blood flow in tumor microvessels;
2. *In vivo* experiments further yielded that a static magnetic field of 0.4 T for 11 days reduced the vascularization and contents of hemoglobin [48];
3. In two independent experiments, nude mice bearing a subcutaneous human colon adenocarcinoma (WiDr) were exposed to 5.5 mT static magnetic fields for 70 min per day; they showed a significant increase in survival time, a significant inhibition of tumor growth, a reduction of cell proliferation and an increase of apoptosis in their tumors [39];
4. Moreover, male Fischer-344 rats that were subjected to 4.5 mT at 120 Hz electromagnetic and magnetic fields; they showed inhibition of preneoplastic lesions through antiproliferative activity of the electromagnetic and magnetic fields, with a decrease of more than 50% of the number and the area of γ-glutamyl transpeptidase-positive preneoplastic lesions, decrease of glutathione S-transferase placental expression, as well as a significant decrease of proliferating cell nuclear antigen, Ki-67, and cyclin D1 [49].

Finally, electromagnetic waves have been used experimentally in synergistic support to chemotherapeutic agents [50,51], and they were shown to decrease the resistance of cancer to chemotherapy [52,53]. Importantly, the electromagnetic waves did not show any toxicity in cancer patients [54,55]; indeed, some data highlight the increase of the survival time of cancer patients with disease progression. The admittedly very rare clinical reports were conducted, for compassionate reasons, in patients with advanced or terminal cancer [55].

However, in contrast to the aforementioned experimental evidence of the effects of low intensity and (low) frequency electromagnetic fields in cancer, there are no data on the mechanism of action [56]. For instance, it has been reported that electromagnetic fields disturb the cancer cells' bioactivity, with a consequent abnormal cell signal transduction process, and that they change the ionic motion (K^+, Na^+, Ca^{2+} and Cl^-) across the cell membrane. Indeed, the oscillating motion of ions near the cell membrane could exert significant voltage fluctuations in the voltage-gated channels leading to a disturbance in the signal transduction process and, consequently, to the inhibition of cell growth [41]. Moreover, several *in vitro* experiments have pointed out that a 1 h exposure to a 50 Hz, 22 mT magnetic field yields an increase in the intracellular Ca^{2+} concentration [57,58], which in turn changes the endonuclease activity. Finally, apoptosis was suggested to be the cause of cell death as a consequence of exposure to electromagnetic fields [59,60]. Regardless, the precise mechanisms by which electromagnetic fields exert their anti-cancer activity are as of yet unclear [61,62].

It is therefore important to note that our entropy generation approach offers a unified theory required to explain these data, starting from the thermodynamic behavior of the cells and linking it to the internal biophysical and biochemical processes.

DISCUSSIONS AND CONCLUSIONS

Since the process of mitosis is considered a promising target in cancer therapeutics, it is fundamental to understand it in more detail in order to develop a comprehensive and ultimately successful treatment strategy. Here, we suggest a thermodynamic approach, based on entropy generation, to analyze the mitosis/apoptosis ratio. The results obtained consist of a mathematical relation between the growth of the tumor mass, and the thermal and geometrical quantities. The graphical representation is the sigmoid 'Gompertz' function [3]; not only does such reproduction of literature data validate our general approach, it also adds some intriguing new considerations:

Starting with equation (10), it follows that the apoptosis probability is related to the geometric factor F_a, which takes into account the finite area of the host tissue/organ. Moreover, the probabilities are related to the forward reaction rate $\dot{\xi}_f$ and to the backward one $\dot{\xi}_b$. It highlights how the reaction rates are fundamental for the behavior of cells. But, these reactions can occur only if the transport of mass across the membrane is successful, i.e., with an efficient ATP-ase. Consequently, it is possible to state that the membrane transport plays a fundamental role in carcinogenesis. So, from the entropy generation analysis of mitosis and apoptosis follows that a potential anticancer (support) therapy can be developed by involving electro-magnetic waves that act on cell membrane behavior. Indeed, Figures 2-3 (a,b) highlight how solid cancer growth can be reduced if subjected to an external magnetic field of the same or one order of magnitude more of the Earth magnetic field: (i) growth control seems to be effective if the field is applied early (Figure 2 vs. Figure 1), echoing the accepted clinical strategy of early cancer detection and rapid start of therapy at smaller tumor masses. Indeed, in the early portion of the Gompertz curve, the cancer mass rapidly increases as mitosis outweighs apoptosis while in the last part of the shape, they balance each other. This notwithstanding, (ii) the percentage reduction of cancer growth at a late stage of the disease (Figure 3 (a)) is particularly impressive, indicating the potential of the external field therapy at advanced cancer stages when conventional clinical strategies are often limited. We note, however, that cancer control is crucially linked to the application of the field as tumor growth ensues once the field is removed, as Figure 3 (b) shows. This points to the benefits of a prolonged treatment strategy with low-level fields [63-69].

In Figure 4, we report our own *in vitro* experimental findings using the triple negative murine breast cancer cell line 4T1, which is a widely employed model to study the behavior of its human homologue cancer type [63]. Indeed this cell line is inducing metastatic spread in BALB/c mice in a manner closely resembling that of the natural occurring human triple negative breast cancer [63]. MTT assay (Roche 11465007001) was performed according to the instruction of the producer. The

yellow tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), is reduced by mitochondria of living cells, therefore dye reduction to blue formazan can be used to estimate cell numbers. The assay is dependent on the ability of viable cells to metabolize a water-soluble tetrazolium salt into a water-insoluble formazan product. The optical density is stable for several hours after solution of the formazan. A linear relationship is seen between optical density and cell number for incubation times of 4h with 20 μL of MTT (5 mg ml^{-1}) added to 200 microliters medium. We have adopted 4 h as the standard incubation time for the assay, absorbance was measured at 595 nm using a Biorad microtiter plates reader. When exposed for 5 consecutive days to a 50 Hz square wave at intensity of 5 μT, the cells showed a reduction of the growth rate (as measured by the tetrazolioum blue assay), compared to unexposed cells, and analogous to the aforementioned reports in the literature [64-70]; similarly, growth inhibition was observed also with exposure to the same frequency, at 245 μT. Lastly, in Figure 5 we then compare the 4T1 cell growth rate, under electromagnetic field treatment, versus our thermodynamic model prediction, using the same field strength. The error between the model and the experimental data is at maximum 0.011%, confirming the good agreement between this novel theoretical approach and the real behavior of cancer growth *in vitro*. Yet, even if it remains an open question whether any of this occurs primarily as a reduction in mitotic rate or through an increase in apoptosis, or if some other mechanism is at work, it is well known that density is preserved in the tumor, with a related higher rate of growth [3]. Consequently, it is important to be able to control the mitosis/apoptosis ratio. In this paper, we suggest to regulate and control this ratio by using a highly innovative strategy: electromagnetic waves. These act on the cell membrane transport, and thus allow us to regulate the mass transport inflow and outflow of the cell. Our aim is to work towards a new approach to anticancer therapy, in support of and as amplifier to present, conventional strategies.

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Figure 1 - The mass variation rate vs. diameter variation for a solid cancer under normal conditions without external field therapy, evaluated using relation (12) with $\kappa = 0$.

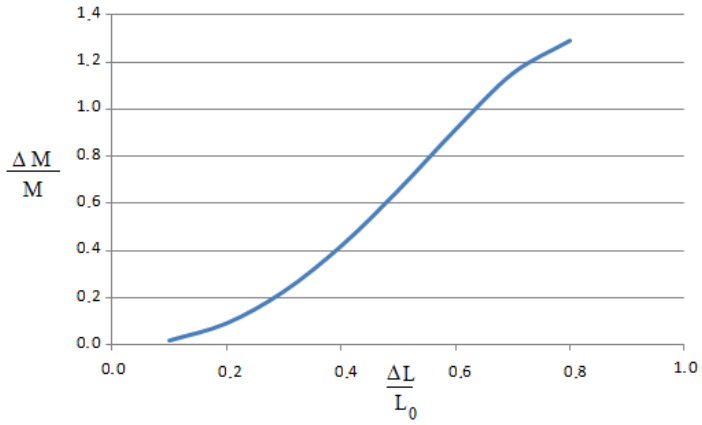


Figure 2 - The mass variation rate vs. diameter variation for a solid cancer under the impact of an external field (for example, a magnetic field of ~ 40 μT with a frequency of ~ 50 Hz), evaluated using relation (12) with $\kappa \neq 0$.

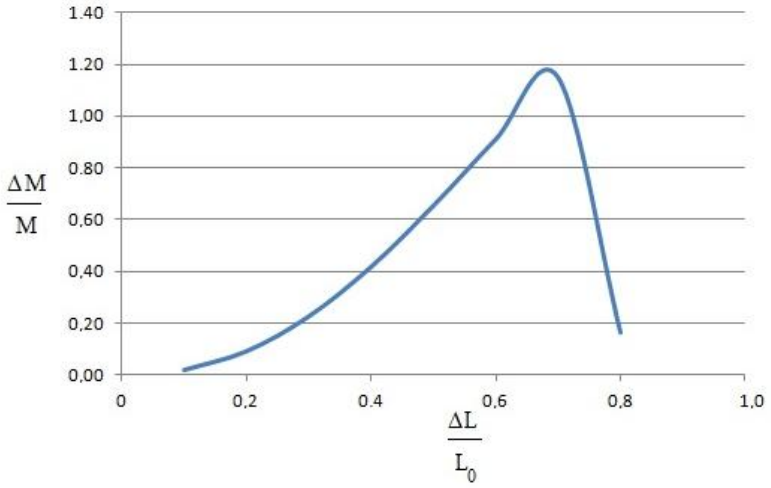
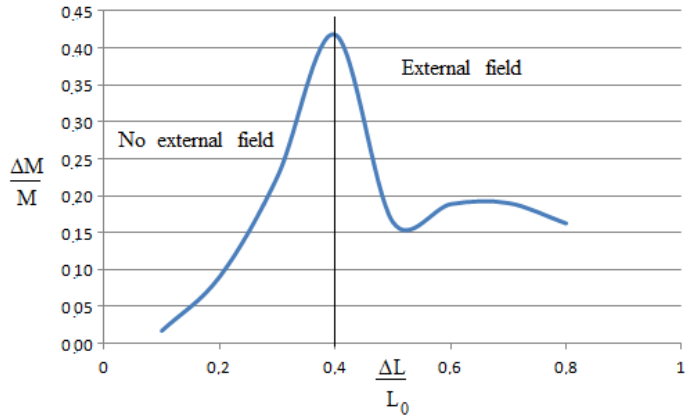


Figure 3 – (a) The mass variation rate vs. diameter variation for a solid cancer under normal conditions for $\Delta L/L_0 < 0.4$ and under the action of an external field (for example, a magnetic field of ~ 40 μT with a frequency of ~ 50 Hz) for $\Delta L/L_0 > 0.4$, evaluated using relation (12) with $\kappa = 0$ for $\Delta L/L_0 < 0.4$ and $\kappa \neq 0$ for $\Delta L/L_0 > 0.4$ at half of the tumor's growth time. (b) Same setup as in (a) but it now depicts the cancer regrowth patterns once the external field is removed.

(a)



(b)

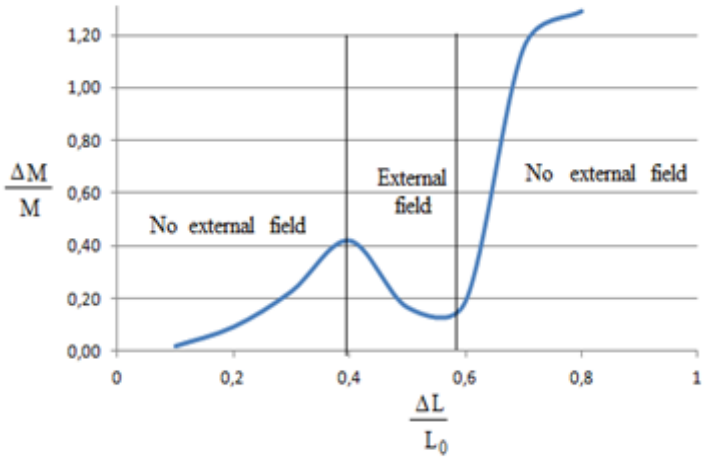


Figure 4 – Depicted is the triple negative murine breast cancer cell line 4T1, exposed over 5 consecutive days to a 50 Hz square wave at intensity of 5 μT (blue; analogous to the simulation set up chosen in Fig. 2), compared with unexposed cells (red). *In vitro* breast cancer growth is partially inhibited by the magnetic field. The quantity OD is the optical density of tetrazolium test in arbitrary units; it is proportional to the number of active mitochondria, hence to the number of alive cancer cells.

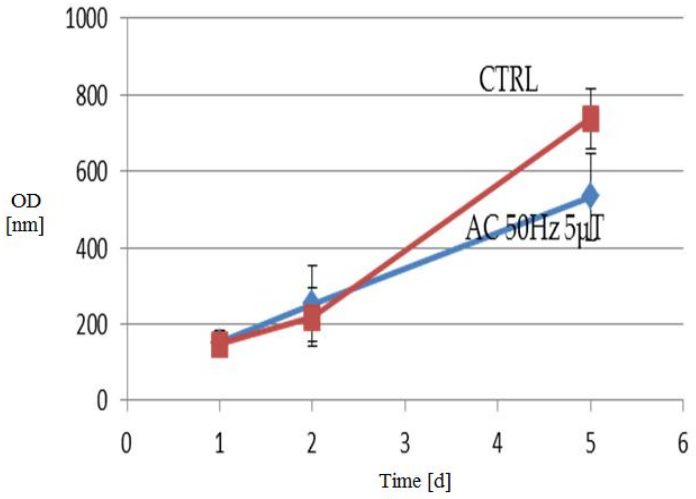


Figure 5 – Shows a comparison between experimental data and our model simulation with the same applied field. Again, the triple negative murine breast cancer cell line 4T1 has been exposed for 5 consecutive days to a 50 Hz square wave at intensity of 5 □T. The maximum error is of the order of 0.011%, thus confirming a good agreement between the theoretical approach and the real behavior of the cancer cells in vitro.