An in-silico Model for Solid Tumor Growth based on the Concept of Glycolysis

M. G. Kounelakis, M. Papadogiorgaki, M. E. Zervakis

***Abstract* — Cancer growth is a complex process which depends on several tasks that cancer cells have to perform in order to live and proliferate. Among these tasks, perhaps the most significant one is to reach adequate sources of nutrients, such as oxygen and glucose, from their surrounding environment in order to initiate their respiration process and provide them with the necessary energy in the form of ATP molecules. Cellular respiration is a biological mechanism that consists of two sequential processes, named 'glycolysis' and 'oxidative phosporylation' (OXPHOS). Since 1956, when the biologist Otto Warburg discovered the increase of glycolysis in cancer cell compared to healthy cells, glycolysis has been studied in depth in order to understand its role in cancer genesis and growth. Towards this direction we propose a new in-silico cancer growth model which embeds the glycolytic potential of the cancer cells in the growth process. The experimental observations obtained show that the model fits the cancer data predicting the tumor's growth, in the proliferative, hypoxic and necrotic zone, quite satisfactory.**

I. Introduction

Cancer is considered as one of the most lethal pathologies. Although it is now clear to scientists that specific genetic mutations are the main cause for carcinogenesis, what it is not yet clear is the mechanism that a solid tumor generates in order to develop and invade.

At tumor's level we now know of three distinct phases that a solid tumor follows in its lifetime. These are the *avascular, the vascular and the metastatic phases*. On the other hand, at tumor cell's level three more zones are recognized [1]-[2]. These are the *proliferative, the hypoxic (or quiescent) and the necrotic zones*, as shown in Fig.1A.

At the proliferative zone, which is the outer zone of the tumor, the tumor cells interact with their surrounding environment to reach necessary nutrients, such as oxygen and glucose, in order to satisfy their energy needs. These energy resources are not unlimited though. This means that there is a moment where oxygen level is below a critical threshold. That time cells suffer from hypoxia and a second zone is created, called hypoxic (or quiescent) zone. Similarly the glucose levels are continuously decreasing which leads to local hypoglycemia. In combination with low level of oxygen, tumor cells start to starve. This condition forces cells to death, which results to the formation of the necrotic core, or the necrotic zone.

Manuscript submitted September 16, 2013.

M. G. Kounelakis, M. Papadogiorgaki and M. E Zervakis are with the Technical University of Crete, Department of Electronic and Computer Engineering.

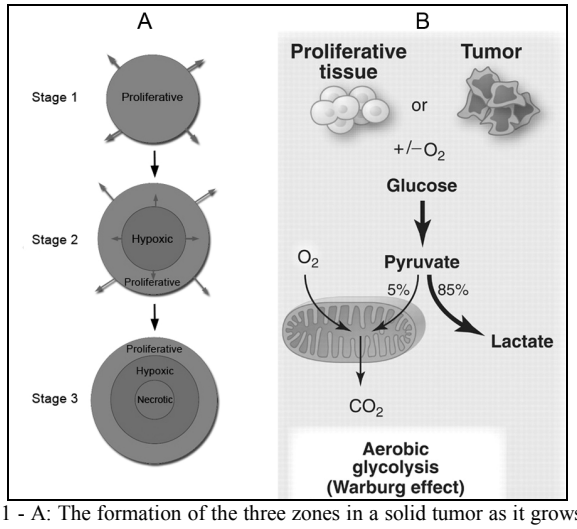

Fig. 1 - A: The formation of the three zones in a solid tumor as it grows. B: The Warburg effect showing that glycolysis in cancer cells is activated even when enough oxygen is present (aerobic glycolysis) [3]-[4].

Current studies focus on the biological processes that a solid tumor adopts in order to grow. In-silico cancer modeling is a research field that promises to assist such research efforts. The synergy of biology, mathematics and computing, but also the fact that a vast amount of patients' data is available now, allows scientists to build such models that can accurately predict the tumor's progressive behavior and most important reveal its future metastatic trends.

Among the biological mechanisms that a solid tumor initiates in order to gain the necessary energy is the glycolysis process. Glycolysis (break down of blood glucose) is the first half of the cellular respiration pathway. The second half is the oxidative phosphorylation (OXPHOS) that takes place in the mitochondrial of the cancer cells. Glycolysis provides only 2 molecules of ATP (energy molecules) while OXPHOS 36 molecules. OXPHOS though starts only if enough amount of oxygen is present.

What is so important with the glycolysis in cancer cells, then?

In 1956 Otto Warburg, a biologist and researcher, noticed that, even if enough oxygen is provided to the tumor, there are many cancer cells that 'prefer' the glycolysis pathway for their energy needs rather than that of the OXPHOS, resulting to high production of lactate (lactate fermentation), as shown in Fig.1B [5]. He supposed that this effect was possibly due to the fact that the mitochondria of the cancer cells have been deactivated or destroyed. This discovery is now known as the *Warburg effect* and since 1956 quite significant research has been done to understand this unusual

978-1-4799-3163-7/13/$31.00 ©2013 IEEE

behavior of the tumor. Towards this direction *enzyme kinetics* were also developed and utilized [6].

Based on the fact that the glycolysis effect directly affects the nutrients supply and consumption inside and outside the tumor, we aim to study the glycolytic potential of the tumor cells, in each one of the three zones (proliferative, hypoxic, necrotic). The final objective is to develop a new in-silico model, which will improve our ability to predict the growth of solid tumors.

II. Materials and Methods

A. Materials

The initial (i.e. at $t = 0$) tumor's size (radius) is taken 1cm = 10mm (5mm for the necrotic zone, plus 3mm for hypoxic zone and 2mm for the external proliferative zone), which corresponds to a clinically detectable size.

The initial values of the nutrients concentrations (O_{vas}, G_{vas}, L_{ex}), the oxygen's and glucose's maximum concentrations (KO_{vas}, KG_{vas}), the extracellular lactate's consumption rate (VL_{ex}), the oxygen's and glucose's maximum uptake rates (VO_{vas}, VG_{vas}), the cells' population carrying capacity (K_m), the proliferation rate (ρ) and the cells' transition rates to hypoxic and necrotic zones ($\alpha_{\mathrm{H}}(\rho), \alpha_{\mathrm{N}}(\rho)$) used in the model are obtained from related literature [7]-[16]. Furthermore, the initial cell populations per zone are as follows: $P_0 = 10^8$, $H_0 = 8\times10^7$, $N_0 = 5.25\times10^7$ for the proliferative, hypoxic and necrotic zone respectively.

B. Methods

Since our model embeds the glycolytic potential of the cancer cells in each tumor zone, it is significant to accurately define this term. Glycolytic potential (denoted as G_{pot}) describes the ability of the cancer cells to reach and consume significant nutrients that are diffused to their environment, either by the vasculature system, which surrounds the tumor (such as oxygen, glucose), or by the cells themselves as products of intracellular biological processes (lactate produced from glycolysis).

As it is understood, the production, diffusion and consumption of these nutrients play a very important role in our model. Based on this fact the well known from chemistry and biology *diffusion-reaction equation* must be adopted [17]. This equation is generally described as the:

(rate of change of nutrient concentration) =

(flux due to diffusion) − (rate of nutrient consumption)

A common practice when designing a mathematical model is to consider specific biological assumptions that can be described by mathematical differential equations. This is done in order to create a simple and understandable model. Following this common rule we defined the biological assumptions for our model, based on the Fig. 2.

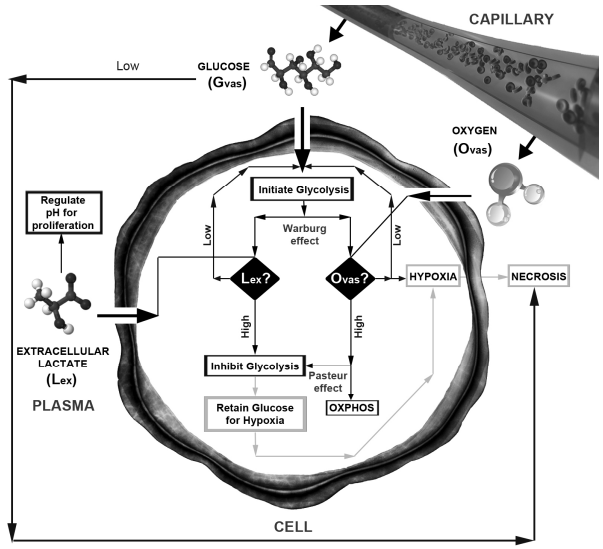

Fig. 2: The intracellular and extracellular diffusion-consumption of oxygen, glucose and lactate and their relation to glycolysis, OXPHOS, pH regulation, hypoxia and necrosis of the cell.

According to Fig.1A and Fig.2 the following assumptions are considered:

Proliferative zone assumptions

- The proliferation zone is increasing due to the increased cell proliferation rate.
- Oxygen and glucose are diffused from capillaries nearby.
- Lactate as a product of glycolysis is diffused to the extracellular area of the tumor.
- Only oxygen and lactate are consumed. Glucose is "*borrowed*" to hypoxic cells by the proliferative cells according to [18].
- Some proliferative cells become hypoxic due to their difficulty to reach oxygen supplies. This means that the rate of cell proliferation is gradually decreasing and the zone reaches its maximum.

The mathematical formulation of the assumptions above is shown in (1) and (2).

$$\frac{dP(t)}{dt} = f(P(t)) - G_{pot}^{P}(t)\cdot P(t) \tag{1}$$

$$G_{pot}^{P}(t) = O_{vas}(t) + L_{ex}(t) - \left(VL_{ex}\frac{VO_{vas}O_{vas}(t)}{KO_{vas}+O_{vas}(t)}\right)\cdot P(t)^2\cdot a_{\mathrm{H}}(\rho) \tag{2}$$

Equation (1) generally describes the proliferative cell population change with respect to the glycolytic potential of the cells in this zone. It contains two terms, the $f(P(t))$ and $G_{pot}^{P}(t)\cdot P(t)$. The factor $f(P(t))$ is described by the second order polynomial equation. Equation (2) describes the glycolytic potential of the cells in this zone witch is a

function of oxygen and lactate diffusion and consumptions. The concentrations of oxygen, glucose and lactate, which vary in time ($O_{vas}(t), G_{vas}(t), L_{ex}(t)$) in (2) and (4) below are estimated from the respective diffusion-reaction equations applied.

Hypoxic (or Quiescent) zone assumptions

- There is no proliferation in this zone.
- It is characterized by increased glycolysis, so only glucose consumption, "*borrowed*" from proliferative cells, is actually observed.
- Lactate production is increased due to increased glycolysis. Lactate then escapes to the proliferation zone as [18] supports.
- This zone is gradually increasing due to the conversion of proliferative cells to hypoxic cells because of oxygen inadequacy.
- But also is gradually decreasing due to the conversion of hypoxic cells to necrotic because of glucose inadequacy.

As before, the mathematics behind these assumptions are presented in (3) and (4). Equation (3) shows the hypoxic cell population change. Equation (4) describes the second term of (3), which shows that the glycolytic potential in the hypoxic region is actually based only on glucose diffusion and consumption. The first term has been already explained before in (2).

$$\frac{dH(t)}{dt} = G_{pot}^{P}(t) \cdot P(t) - G_{pot}^{H}(t) \cdot H(t) \qquad (3)$$

$$G_{pot}^{H}(t) = G_{vas}(t) - \left(\frac{VG_{vas}G_{vas}(t)}{KG_{vas} + G_{vas}(t)} \right) \cdot H(t) \cdot a_{\mathrm{N}}(\rho) \qquad (4)$$

Necrotic zone assumptions

- Again as in hypoxic zone there is no proliferation of cells.
- There is no nutrients consumption but only nutrients diffusion.
- The zone is increasing due to conversion (or apoptosis) of hypoxic cells to necrotic because of glucose inadequacy.

The mathematical formula which describes the necrotic cell population change is shown in (5).

$$\frac{dN(t)}{dt} = G_{pot}^{H}(t) \cdot H(t) \qquad (5)$$

The novelty of this model is based on: a) the glycolytic behavior of each cancer zone, b) the glycolytic potential of each cancer cell and its influence on the growth of the zone it belongs and c) the interaction between the three zones due to the transition of the cancer cells from one zone to another.

The compartmental equations of the proposed model along with its solution have been numerically approximated by means of a finite difference method, namely Forward Euler.

III. Experimental results

The model was tested for its ability to predict the tumor's growth (expressed in cell population change) within each zone. For this purpose three different proliferation rates (ρ) were considered, which are 0.12/day, 1.0/day and 5.2/day as proposed in related literature [7]. The simulation time was 1 year (or 360 days). The testing procedure, the parameter selected and the simulation results were according to medical standards and approved by clinical experts.

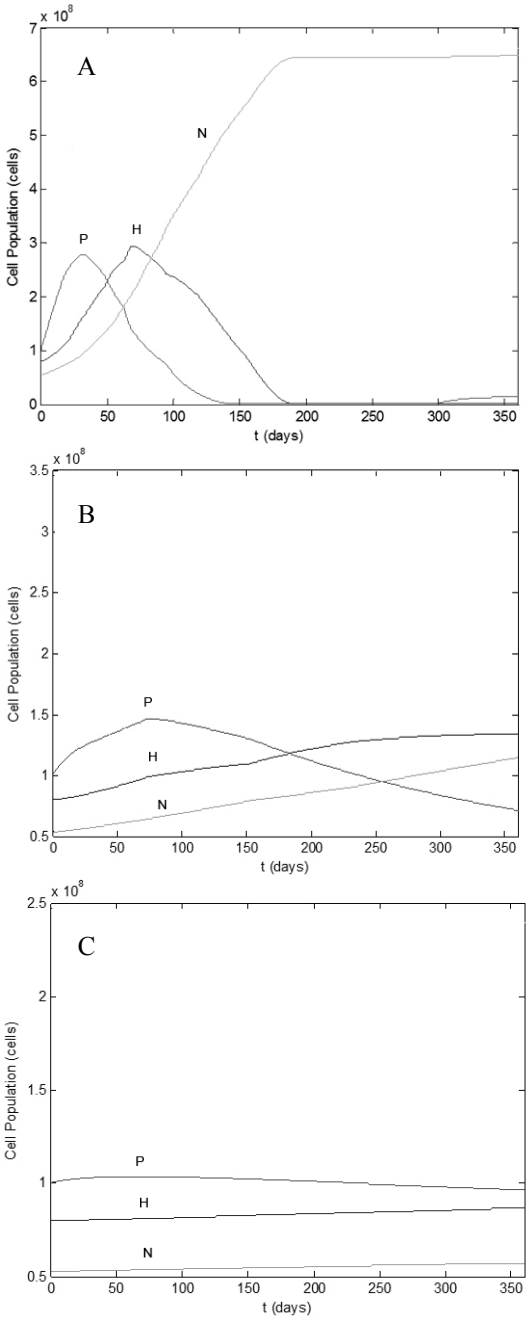

Fig. 3: Solid tumor's growth within each zone for a time period of 360 days under three different proliferation rates. Symbols for zones - P: proliferative, H: Hypoxic, N: Necrotic. A: Tumor's growth at $\rho = 5.2/day$. B: Tumor's growth at $\rho = 1.0/day$. C: Tumor's growth at $\rho = 0.12/day$.

IV. DISCUSSION

Observing Fig.3A we can easily notice that when the proliferation rate ρ reaches the highest value of 5.2, the proliferative and hypoxic zones are increasing with certain growth rates. After a time period of 30 days however, the proliferative zone starts to decrease due to transition of its cells to the hypoxic zone because of the insufficient sources of oxygen and lactate. This fact inevitably leads to the increase of the hypoxic cell population that continues to increase until the 70^{th} day. From this point on until the 180^{th} day the hypoxic zone is decreasing due to the lack of sufficient amount of glucose and the decreased rate of glycolysis. As far as the necrotic zone concerns, we can notice that there is a rapid growth until the 180^{th} day due to the quick depletion of oxygen and glucose within the other two zones. After that day, it appears that the necrotic zone reaches its limit, which means that there is no further significant growth. This is the time when the entire tumor reaches its steady state.

In Fig. 3B, we present a similar tumor's growth pattern. The proliferation rate is now lower (1.0) and we notice that again the proliferation zone is growing until it reaches an upper limit, but this time at a longer time period of 80 days. This is expected due to lower proliferation rate. Furthermore, both hypoxic and necrotic zones follow almost the same smooth growth pattern until the end of the year (i.e. at 360 days), while the proliferative cell population is constantly decreasing.

Finally Fig. 3C presents the case of a proliferation rate even lower (0.12). We observe that both the proliferative and hypoxic zones do not change dramatically, because the transition of proliferative cells to the hypoxic zone happens in a much slower rate. Accordingly, the necrotic zone remains almost unchanged, implying that at the end of the first year the nutrient supplies have not yet been exhausted.

V. CONCLUSIONS

Since 1956 where Warburg effect was documented, glycolysis has been considered as one of the metabolic pathways with great influence on tumor's behaviour.

The present study examines the impact of glycolysis on a developed (1 cm radius) solid tumor growth. This is achieved with the implementation and testing of an in-silico model, which embeds the glycolytic potential of tumor cells in each one of the three different zones. We demonstrate that glycolysis is directly related to the diffusion-consumption of nutrients that in turns greatly affects tumor's growth behaviour. This is justified from the fact that the model adopts the Warburg and the Pasteur effects and the recent Sonveaux et al observation [18]. Our study further explores the effects of glycolysis in different stages of cancer, with an aim to contributing towards personalised medicine.

ACKNOWLEDGMENT

Present work was supported by YPERTHEN, Interreg Greece-Cyprus 2007-2013, funded project by National Strategic Reference Framework (NSRF) 2007-2013.

REFERENCES

[1] H. P. Greenspan, "Models for the growth of a solid tumor by diffusion", *Studies in Applied Mathematics*, vol. LI, pp. 317-340 (1972).

[2] T. Roose, S. J. Chapman and P. K. Maini, "Mathematical Models of Avascular Tumor Growth", *Society for Industrial and Applied Mathematics*, vol. 49, pp. 179-208, (2007).

[3] M. G. Vander Heiden, L. C. Cantley and C. B. Thompson, "Understanding the Warburg effect: the metabolic requirements of cell proliferation", *Science*, vol. 324, pp. 1029-1033, (2009).

[4] P. Ampatzoglou, "Computational modeling of cancer tumor growth", Open Education - *The Journal for Open and Distance Education and Educational Technology*, vol. 8, No. 1, (2012).

[5] O. Warburg, "On the origin of cancer cells", *Science*, vol. 123, pp. 309–314, (1956).

[6] L. Menten and M. I. Michaelis, "Die Kinetik der Invertinwirkung", *Biochemische Zeitschrift*, vol. 49, pp: 333–369, (1913).

[7] A. C. Burton, "Rate of Growth of Solid Tumors as a Problem of Diffusion," *Growth*, vol. 30, pp. 229–244, (1966).

[8] J. P. Freyer and R. M. Sutherland, "Regulation of Growth Saturation and Development of Necrosis in EMT6/Ro Multicellular Spheroids by the Glucose and Oxygen Supply", *Cancer Research*, vol. 46, pp. 3504–3512, (1986).

[9] J. J. Casciari, S. V. Sotirchos and R. M. Sutherland, "Glucose diffusivity in multicellular tumor spheroids", *Cancer Research*, vol. 48, pp. 3095- 3099, (1988).

[10] J. J. Casciari, S. V. Sotirchos and R. M. Sutherland, "Mathematical modeling of microenvironment and growth in EMT6/Ro multicellular tumor spheroids", *Cell Proliferation*, vol. 25, pp. 1-22, (1992a).

[11] J. J. Casciari, S. V. Sotirchos and R. M. Sutherland, "Variations in tumor cell growth rates and metabolism with oxygen concentration, glucose concentration, and extracellular pH", *Journal of cellular physiology*, vol. 151, pp: 386-394, (1992b).

[12] J. P. Freyer and R. M. Sutherland, "A Reduction in the In Situ Rates of Oxygen and Glucose Consumption of Cells in EMT6/Ro Spheroids During Growth", *Journal Of Cellular Physiology*, vol. 124, pp. 516-524, (1985).

[13] K.L. Kiran, D. Jayachandran and S. Lakshminarayanan, "Mathematical Modelling of Avascular Tumour Growth Based on Diffusion of Nutrients and Its Validation", *The Canadian Journal Of Chemical Engineering*, vol. 87, pp. 732-740, (2009).

[14] M. G. Nichols and T. H. Foster, "Oxygen diffusion and reaction kinetics in the photodynamic therapy of multicell tumour spheroids", *Physics in Medicine and Biology*, vol. 39, pp. 2161-2181, (1994).

[15] V. Busini, P. Arosio and M. Masi, "Mechanistic Modelling of Avascular Tumor Growth and Pharmacokinetics Influence—Part I", *Chemical Engineering Science*, vol. 62, pp. 1877–1886, (2007).

[16] K. R. Swanson, R. C Rockne, J. Claridge, M. A. Chaplain, E. C. Alvord Jr and A. R. Anderson "Quantifying the role of angiogenesis in malignant progression of gliomas: in silico modeling integrates imaging and histology", *Cancer Research*, vol. 71, pp. 7366-7375, (2011).

[17] R. A. Gatenby and E. T. Gawlinski, "A reaction-diffusion model of cancer invasion", *Cancer Research*, vol. 56, pp: 5745-53, (1996).

[18] P. Sonveaux, F. Végran, T. Schroeder, M. C. Wergin, J. Verrax, Z. N. Rabbani, C. J. De Saedeleer, K. M. Kennedy, C. Diepart, B. F. Jordan, M. J. Kelley, B. Gallez, M. L. Wahl, O. Feron and M. W. Dewhirst, "Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice", *The Journal of Clinical Investigation*, vol. 118, pp. 3930-3942, (2008).