Validation of MRS metabolic markers in the classification of brain gliomas and their correlation to energy metabolism

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Abstract- **The aim of this study is to validate the significance of recently identified MRS (Magnetic Resonance Spectroscopy) ratio-type metabolic markers used in brain gliomas classification, through the energy metabolism profile of these complex tumors. It is an attempt to integrate the metabolic knowledge extracted from MRS analysis of patient gliomas provided, with proteomic knowledge derived from metabolic enzymes that participate in the energy production process, called glycolysis.**

The results indicate that the increased levels of lactate, alanine and fatty acids measured from MRS spectra in gliomas are justified from the behavior of metabolic enzymes confirming thus the fact that the ratio-type metabolic markers are highly significant for the discrimination of such brain tumors.

Keywords- **Energy metabolism, glycolysis, brain gliomas**

I. Introduction

Malignant rapidly-growing tumor cells, including brain gliomas, typically have very high glycolytic rates compared to their counterparts in normal tissue. There are two common explanations for this fact. The classical explanation is that there is poor blood supply to tumors causing local depletion of oxygen. The other explanation stems from the well known hypothesis of Otto Warburg, who claimed that most cancer cells predominantly produce energy by glycolysis followed by lactic acid fermentation in the cytosol, rather than by oxidation of pyruvate in mitochondria like most normal cells [1]. This occurs even if oxygen is plentiful. Warburg postulated that this change in metabolism is the fundamental cause of cancer [2], a claim now known as the Warburg effect. This effect may simply be a consequence of damage to the mitochondria in cancer, or an adaptation to low-oxygen environments within tumors, or a result of cancer genes shutting down the mitochondria because they are involved in the cell's apoptosis program which would otherwise kill cancerous cells. The Warburg effect may also be associated with cell proliferation. Since glycolysis provides most of the building blocks required for cell proliferation, it has been proposed that cancer cells (and normal proliferating cells) may need to activate glycolysis despite the presence of oxygen in order to proliferate [3].

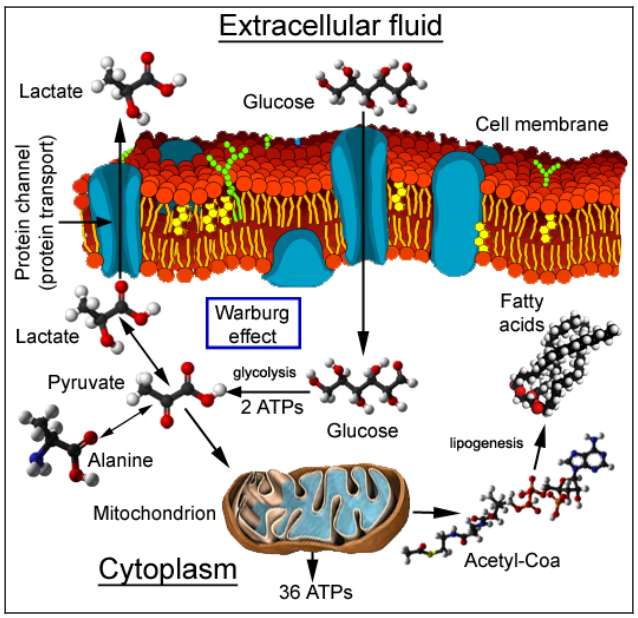

Fig. 1. Glycolysis and lipogenesis processes

Glycolysis (a sugar splitting process), as shown in Fig. 1, involves a series of biochemical reactions in which glucose is broken down to pyruvate with the release of usable energy in the form of ATP (adenosine triphosphate) molecules. Under aerobic conditions, the dominant product in most tissues is pyruvate and the pathway is known as aerobic glycolysis. When oxygen is depleted, as for instance in hypoxic-necrotic tumorous tissues of gliomas, the dominant glycolytic product in many tissues is lactate and the process is known as anaerobic glycolysis. Thus, lactate metabolite is a sensitive indicator of anaerobic glycolysis and reduced cellular oxygenation in living tissues. Along similar lines alanine, in conjunction with lactate, increases

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in tissues during hypoxia; made by transamination of pyruvate to prevent further increases in lactate [4]. Finally, fatty acids (lipids) are an important source of energy too. Excess glucose can be stored efficiently as fat. All cell membranes are built up of phospholipids, each of which contains fatty acids [5].

Therefore, reliable estimates of the levels of lactate, alanine and lipid resonances as exhibitors of glycolysis are of special interest for the clinical management of brain gliomas patients. The metabolic pathway of glycolysis is a series of chemical reactions catalyzed by specific metabolic enzymes. These glycolytic enzymes have direct relation to the metabolites mentioned above.

The main goal of this study is to correlate the knowledge derived from these glycolytic enzymes with the information derived from the statistical analysis of the above mentioned metabolites, thus validating the diagnostic importance of ratio-type MRS markers, recently identified [6,7], wherein these metabolites dominantly participate.

II. Materials and Methods

Among the glycolytic enzymes that participate in the energy production process, necessary both for the healthy and cancerous cells, the HK-Hexokinase (EC 2.7.1.1), PK-Pyruvate Kinase (EC 2.7.1.40), and LDH-Lactate Dehydrogenase (EC 1.1.1.27) are the most significant, as it can be observed from the glycolysis metabolic pathway presented in KEGG (Kyoto Encyclopedia of Genes and Genomes) and BRENDA (The Comprehensive Enzyme Information System) enzyme databases. The EC number corresponds to the Enzyme Commission classification.

HK is the enzyme that catalyzes the first reaction in glycolysis pathway, the glucose conversion to pyruvate, even when blood glucose levels are relatively low. PK catalyzes the last step of glycolysis, in which pyruvate and ATP are formed. Finally LDH converts pyruvate to lactate when oxygen is absent or in short supply (anaerobic process).

The strategy followed in order to reveal the interrelation of these enzymes with the metabolites mentioned in the introduction, consists of two steps:

1. Identify (through literature search) the bioenergetic activity of each one of the glycolytic enzymes in gliomas and,
2. Relate this activity with the metabolic behaviour of the pyruvate, lactate, alanine and fatty acids (lipids) by measuring their peak-area levels in a given dataset of short echo magnetic resonance spectroscopy imaging (MRSI) data from 21 glioma patients.

The dataset provided consists of short echo magnetic resonance spectroscopy imaging (MRSI) data from 21 glioma patients, as presented in Table 1. The two-dimensional MRSI data was collected by the Radboud University and contains 303 pre-processed ^{1}H-MRSI volume elements (voxels) corresponding to 303 spectra. Each patient case had passed strict quality control and validation procedures, including consensus histopathologic determination.

Table 1 Analysis of the MRSI dataset
GR2: glioma grade 2, GR3: glioma grade 3 and GR4: glioma grade 4

Tissue type	No of subjects	No of voxels
GR2	10	176
GR3	4	57
GR4	7	70
	Total	303

The peak areas, Fig. 2, obtained by peak integration of pyruvate (at 2.37 ppm), alanine (at 1.48 ppm), lactate (at 1.33 ppm) and lipids resonances (at 0.90 and 1.30 ppm) [7].

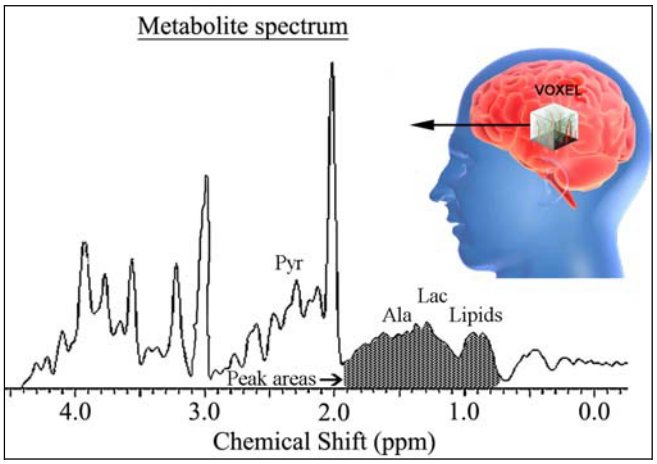

Fig. 2. Spectrum obtained from a voxel. Y axis: peak heights (proportional to metabolites concentration). X axis: frequency (position) in parts per million. Pyr (Pyruvate), Ala (Alanine), Lac (Lactate), Lipids (mobile lipids) are the metabolites observed. The shaded area corresponds to the areas under the peaks

III. Results

Following the above-mentioned strategy, an investigation of recent and past studies involving the glycolytic activities of these 3 enzymes has been performed in order to record their metabolic behaviour in brain gliomas. The bioenergetic activities of these enzymes are presented in Table 2. It can be observed that the activity of

the enzymes increases as the tumor becomes more malignant [8-10].

Table 2 Bioenergetic activities of HK, PK and LDH in gliomas

Tissue type	HK	PK	LDH
GR4 GR3 GR2	Rise	Rise	Rise

The following step is to measure, in the 3 classes of Table 1, the mean values of the areas under the peaks of the pyruvate, lactate, alanine and lipids from the given MRSI dataset. The comparison of the mean values is shown in Fig. 3 below. The most important observation in this figure is the rapid increase of both lactate and lipids in GR4 compared to their levels in GR3 and GR2. This fact underlines the lack of oxygen in this tumorous tissue. Alanine and pyruvate are also elevated as tumor grade increases, but their levels are considerably lower than those of lactate and lipids.

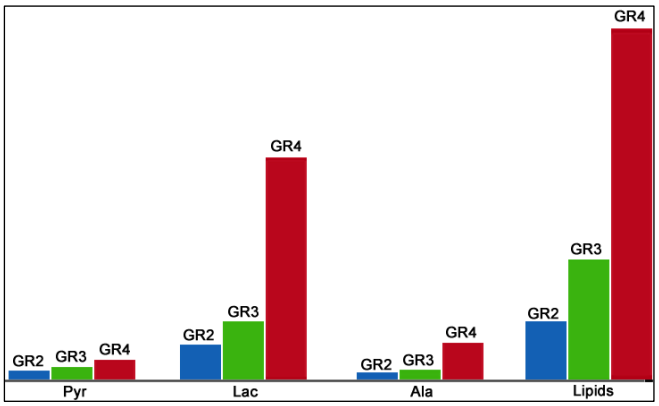

Fig. 3. The mean peak-area values of the 4 metabolites measured in the 3 classes provided.

Furthermore, we estimate the statistical significance of the differences of these means. These observations are presented at Table 3. Relating the enzymes activities of HK, PK and LDH with the statistical findings of the MRS metabolites levels of Table 3, we can deduce important interactions which are explained in the Discussion section.

Table 3 The mean values of the 4 metabolites peak-areas
HS: p value < 0,01 – S: p value < 0,05

Metabolites / Classes	Low grade	Intermediate	High grade
Pyruvate	GR2	GR3	GR4
	GR2	S	S
	GR3		S
	GR4		
Lactate	GR2	GR3	GR4
	GR2	HS	HS
	GR3		HS
	GR4		
Alanine	GR2	GR3	GR4
	GR2	S	HS
	GR3		HS
	GR4		
Lipids	GR2	GR3	GR4
	GR2	HS	HS
	GR3		HS
	GR4		

IV. Discussion

As mentioned in the introduction, brain tumors and gliomas, in particular, develop high glycolytic rates. This phenomenon has an intracellular impact to the glycolytic enzymes' activities that control the ATP energy molecules or in other words the energy flux in the cell [11]. Due to this fact the HK enzyme activity is increased as shown at Table 2. This behavior is justified from the fact that HK, particularly the HK-II isoform, plays a critical role in initiating and maintaining the high glucose catabolic rates of rapidly growing tumors [12].

PK as the last enzyme of the glycolytic chain also increases with tumor grade [9] as shown in Table 2. This activity forces the levels of pyruvate as an end glycolytic product to rise accordingly, as shown in Fig. 3. Furthermore and dependent on the energy needs, the pyruvate metabolite is converted to either lactate or lipids. Lactate is an energy rich molecule which, given some oxygen, can be also converted to pyruvate and so enter the mitochondria to generate a bucket load of ATP but also lipids, as shown in Fig. 1. In highly hypoxic-necrotic areas of the tumor cells, like in GR4, the brain neurons do not use glucose at all, glucose is converted to lactate by the astrocytes and it is lactate which feeds directly in to the neuronal mitochondria via pyruvate. So lactate with oxygen is a potent combination for ATP generation. In the aerobic bulk of the tumour glucose can be burned via pyruvate in the mitochondria and there is no need for lactate production.

Lactate dehydrogenase (LDH) is also a key metabolic enzyme catalyzing pyruvate into lactate and is excessively expressed by tumor cells [10, 13] causing an increase in the lactate levels as shown in Fig. 3 too.

Furthermore, alanine is also produced during hypoxia by transamination to pyruvate from another amino acid [4].

Pyruvate levels when compared with those of lactate and lipids in the three types of tumors vary significantly as shown in Fig. 3. This is expected since pyruvate is

immediately converted to either lactate after the glycolytic process (Warburg effect) through the fermentation process or lipids through the lipogenesis process. Then, lipids and lactate metabolites levels increase rapidly as the malignancy increases. The highly statistical differences in their mean values also prove this tendency. Furthermore, Alanine's mean values also present a highly significant difference between GR2 and GR4 but also between GR3 and GR4, due to the hypoxia observed in high grade tumors.

Based on these observations and the fact that lactate, alanine and lipids metabolites are the main regulators of the energy flux within the cancerous cells we easily conclude to the fact that they should be significant in the discrimination of gliomas types and grades too. Since glycolysis is of vital importance in brain cancer cells survival and proliferation, understanding of the metabolic activities of the enzymes mentioned and their related metabolites, in different grades of gliomas, can help us identify reliable markers for diagnostic purposes.

Previous research accomplished by our team has proved the significance of these metabolites in gliomas discrimination [6, 7]. In these studies we have shown that peak area ratio-type markers who involve lactate, alanine and lipids play a crucial role in grade and type classification of such complex tumors. More specifically the ratio markers of Lac/Cre, Ala/Cre, Ala/S, Lips/Cho and Lips/Cre, which have been found to provide a classification accuracy of 84% in GR2 vs GR3 gliomas. Furthermore the S variable used as denominator in this binary classification but also in the GR3 vs GR4 and GR2 vs GR4 includes the peak area levels of the metabolites.

V. Conclusions

The study of bioenergetics in gliomas is a very promising field for clinical and biological management of complex brain tumors. Since the Warburg hypothesis, a lot of research has been directed towards identifying how the metabolic enzymes' activities and their relation to the expression of certain metabolites, related to glycolysis and mitochondrial respiration, affect tumor cell survival and proliferation.

Adopting this hypothesis in this study we attempt to identify the way in which important metabolic enzymes such as HK, PK and LDH are related to the metabolic behavior and functionality of lactate, alanine and lipids in 3 different types of gliomas in a dataset of 21 patients. The significant influence of these metabolites in gliomas classification was also confirmed by recent studies of our team where it is clear that diagnostic markers that contain these metabolites provide high classification accuracies.

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