RESEARCH STUDIES

Energetic metabolism of glioma stem cells

D. Lisii

Institute of Neurology and Neurosurgery
2, Korolenco Street, Chisinau, Republic of Moldova
Corresponding author: dlisii@yahoo.fr. Manuscript received October 23, 2012; revised December 15, 2012

Abstract

Tumor cell energy metabolism is often invoked as marker of aggressiveness and resistance in the study of cancer. Warburg was the first researcher to propose the high consumption of glucose and reduction of tumor cell oxidative metabolism as prominent feature of cancer cells. In clinical practice, this feature is used in PET imaging (positron emission tomography) to visualize the 2-FDG antimetabolite enhancing lesions. In reality, not all tumors are characterized by high levels of glycolysis. To highlight certain features affected by glucose and energy, four types of cancer stem cells (CSCs) were isolated from the third degree of malignancy gliomas (anaplastic glioma, WHO, World Health Organization). The NADH cells' levels of CSCs in sphere cultures where evaluated and compared to a proliferation index. Surprisingly, the cells with the highest proliferation index showed a lowest ratio of NADH/proliferation. Thus, the energy status of cancer stem cells lines derived from gliomas is inversely correlated to proliferation in vitro. Glucose dependency varied considerably among the four types of glioma stem cells. In one case, NADH levels were maintained in the absence of glucose by substitution with glutamine. DCA (dichloroacetat) is an energy modulator acting as a mitochondrial function stimulator. In our experiments, DCA could stimulate NADH production, showing the possibility of mitochondrial function recovery in glioma cancer stem cells.

Key words: cancer, glioma stem cells, tumor cell energy metabolism.

Энергетический обмен стволовых клеток глиомы

В исследовании агрессивности и сопротивления рака часто используются особенности энергетического метаболизма опухолевых клеток. Варбург был первым, кто поставил акцент на высокий уровень потребления глюкозы и снижения окислительной функции опухолевых клеток. В клинической практике эта функция используется в ПЭТ (позитронно-эмиссионной томографии), сканирование с помощью метаболит 2-FDG. На самом деле, не все опухоли можно охарактеризовать высоким уровнем гликолиза. Для характеристики зависимости от глюкозы и выделения некоторых энергетических особенностей были выделены из глиомы четыре типа стволовых клеток, с сопоставимой степени злокачественности (3 anaplastic – 3 анапластической, Всемирная Организация Здравоохранения). Удивительно, что стволовые клетки с высоким индексом пролиферации показали самый низкий уровень NADH (энергетический эквивалент снижения). Энергетический статус нормальных нервных стволовых клеток, вызванный NADH, был самым высоким. Зависимость от глюкозы значительно различалась между четырьмя типами стволовых клеток глиомы, а в одном случае, уровень NADH был сохранен в отсутствие глюкозы при замещении глутамина. При лечении с DCA (dichloroacetat) производства NADH можно стимулировать, показывая вожможность частичного восстановления митохондриальной функции опухоли.

Ключевые слова: рак, глиома, энергетический обмен стволовых клеток.

Introduction

Gliomas are represented by a heterogeneous group of tumors and made up essentially by two main populations of glial cells: oligodendrocytes and astrocytes. Cancer stem cells can be isolated from particular tumors and are thought to be the cause of chemo- and radiotherapy resistance and tumor relapse. The main data concerning tumoral in vivo energetic metabolism derives from spectroscopic imagistic data SPET (positron emission tomography) utilizing 2-FDG (fluoro-deoxy-D-glucose) and methyonine as a tracer [2, 3, 4]. Based on these results, it was widely recognized that some cancer cell types show enhanced glycolysis levels [1, 10]. High glucose turnover and lactate production might represent an adaptive mechanism in response to increasing hypoxia accompanying tumoral progression. Moreover, resulted peritumoral acidosis is regarded as one of tumoral aggressive factors for surrounding normal cells. A reduced spatial and temporal resolution of metabolic studies via PET scan imagery is incriminated by the impossibility to evaluate specific metabolic profiles for each cell type involved in tumoral formation.

In an attempt to establish the correlation between glioma CSCs energetic metabolism and tumoral aggressiveness, four types of glioma stem cells were isolated from tumors originating from the brain stem, thalamus, and hemispheres. Energetic metabolism of spheroids of CSCs evaluated by NADH levels depending on glucose levels in cultural media and treated with antimetabolite 2DG in some conditions was compared with neural stem cells originating from a neonatal mouse.

Material and Methods

Four cancer stem cell lines were isolated using classical methods. 2 adult glioma cells originated from the thalamus and frontal lobe for which an anatomopathologic study revealed an anaplastic glioma grade III of malignancy according to the WHO classification, and 2 child anaplastic gliomas from brain stems with similar degree of malignancy. Each type of glioma stem cell was cultured and maintained in an atmosphere with similar CO2 and O2 contents. Different types of cell culture media were used for adult and child stem

cells gliomas to assure best proliferation rates. TP54 and TP84 stem cell lineages from the adult glioma were cultured in a "house" prepared D3 media: DMEM-F12 1X liquid 10 ml, Glucose 30% 200 µl. (50.8 mM), Glutamine 200 mM 100 µl (2 mM), Penicillin Streptomycin 100 µl g/ml 10 µl (0.1 mkg), with a neural stem cell supplement 50 X 200 µl, Heparin 20 mg/ml 0.5 µl (20 mg/ml), EGF (epidermal growth factor) 1 µl (20 ng/ml), bFGF 1 µl (basic fibroblast growth factor) (10 ng/ml). TG10 and OB1 stem cell lineage derived respectively from child brain stem glioma was cultured in a NS34 media: N2100 x and B27 50 X, G5100 x 1 ml (Stem Cell). The SCN24 neural stem cells from the mouse were cultured in a standardized media from Stem Cell.

In order to test each stem cell line dependence on main nutrients, each cell lineage was resuspended in the same D3 culture media with known concentrations of Glucose and Glutamine for 24 hr. prior to glucose and glutamine deprivation. NADH or NADPH "reducing equivalents" as markers of energetic metabolism in viable cells has been quantified by WST test (Roche). The same number per well were seeded (20.000) and the cell viability essay was performed by Blue-Trepan exclusion method.

In the second essay, we treated our glioma stem lines and neural stem cell lines with several modulating energy metabolism agents: DCA (dichloracetate), which accelerates the Krebs cycle by inhibiting the activity of PDK (pyruvate dehydrogenase kinase); 2 DG (2 deoxy-D-glucose) an analog of glucose that blocks the first step of glycolysis; Antimycine A - a mitochondrial inhibitor which is involved in the energy-coupling site of the respiratory system by blocking the electrons flow from cytochrome b to cytochrome c1.

Results

Under the same cultured condition (white column) the NADH activity was greater for stem cell lines derived from child brain stem glioma. The dark column represents NADH activity when cultured in the original media. Interestingly, the proliferative index (data not shown) was greater for the stem cell line derived from the adult brain glioma, thus the cell proliferation rates inversely correlated with metabolic NADH activity (fig. 1).

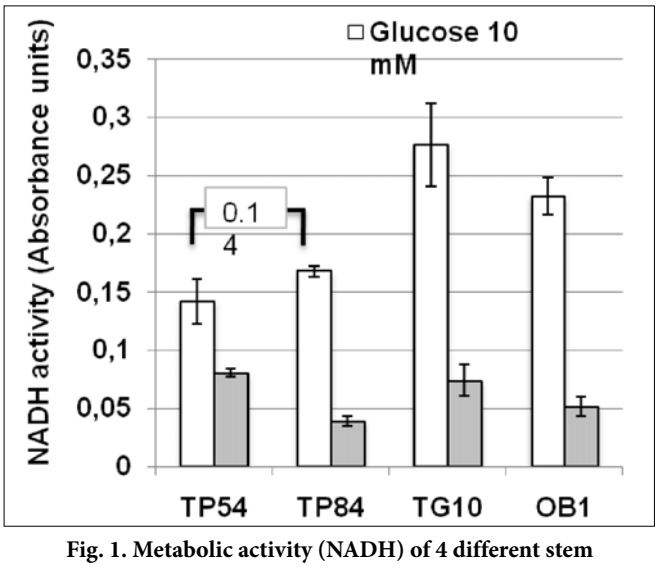

Fig. 1. Metabolic activity (NADH) of 4 different stem cell lines in cultured media.

Great differences were revealed in the metabolic activity of stem cell lines derived from morphologically similar grade III (WHO) gliomas. The TP54 cell line maintained the same activity of NADH with Glutamine 2 mMol in the media as with Glucose 10 mMol, demonstrating the great adaptability of TP54 compared withTP84 (fig. 2).

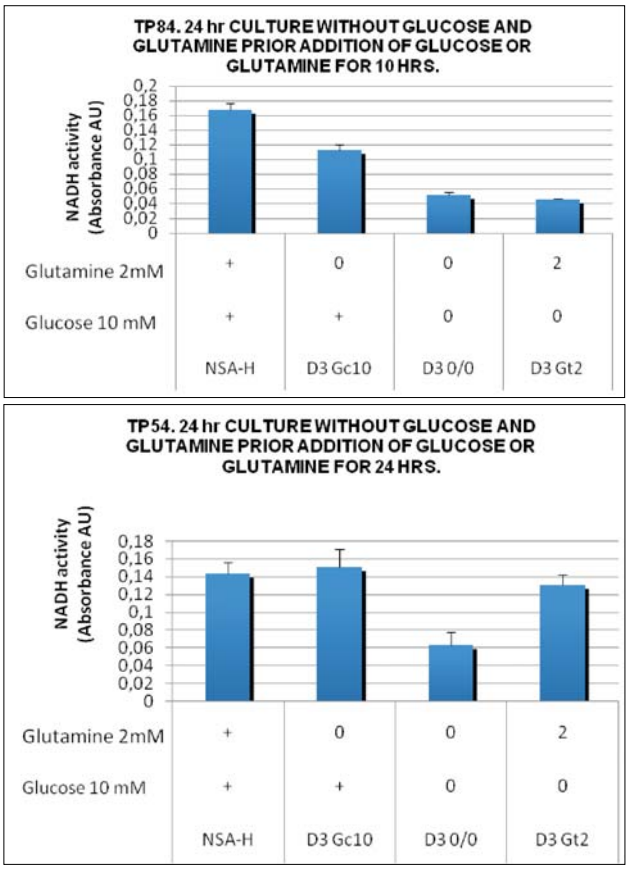

Fig. 2. Differences in stem cell dependency on Glucose versus Glutamine for NADH production.

The CSN24 mouse neural stem cell line treated with 2DG (2-deoxy glucose) shows little or no blockage of NADH activity, indicating both a low cell glycolytic activity and a relative resistance to 2DG antimetabolite (fig. 3).

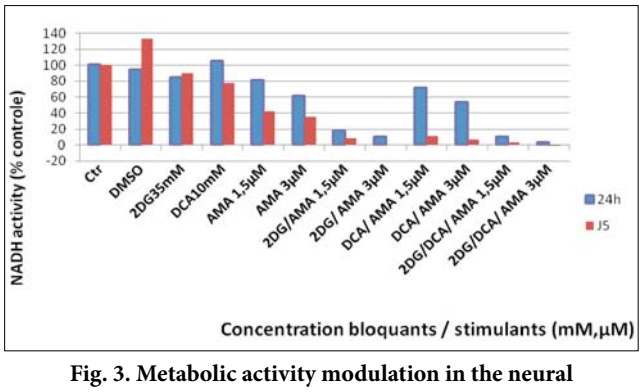

Fig. 3. Metabolic activity modulation in the neural stem cell line.

The TG10 stem cell line derived glioma from child brain stems treated with 2DG shows a net reduction of metabolic activity compared to CSN24. In contrast, DCA stimulated Krebs cycle activity, confirming an inhibited status of mitochondria in cancer (fig. 4).

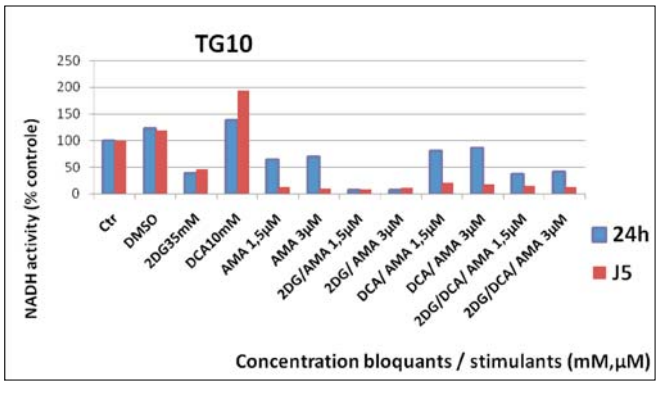

Fig. 4. Stem cell lines derived from glioma TG10; in conditions with two antimetabolite 2DG (2-deoxyglucose) and AMA-Antimycine A, and stimulating drug DCA (dichloracetate).

Discussion

"Cancer cells originate from normal body cells in two phases. The first phase is the irreversible injuring of respiration. The irreversible injuring of respiration is followed, as the second phase of cancer formation". (On the origin of cancer cells, Otto Warburg, 1956).

It has previously been emphasized that, beyond its impact in terms of diagnosis, growth patterns analysis, and aberrant energetic metabolism evaluation provide valuable insights into the clinical manifestation and imaging of tumors. Several studies demonstrate the role of PET scan imaging based on high glycolysis cells on tumoral diagnosis and prognostic evaluation [2, 7]. Thus, the 2-FDG PET scan (fluoro-deoxy-glucose positron emission tomography) is largely utilized in clinics for evaluation of local and distant lymph node metastasis. A metabolic study of FDG PETs shows a better specificity and sensitivity compared to the conventional CT (computer tomography) and MRI (magnetic resonance imaging) techniques with 79-85% and 90-99% respectively [2, 9]. Finally, the great resolution of the FDG PET scan makes it a first choice exam in some cancers type: head and neck, breast, lung, colorectal, melanoma and lymphoma tumors [3, 4, 5]. Energetic metabolism and some key enzymes particularly implicated in glycolysis and oxidative phosphorilation have been studied as potential markers of tumoral aggressiveness. For example, based on immunohystochemical evaluation of GAPDH (glyceryl aldehyde phosphate dehydrogenase), beta-1 ATPase subunit (adenosine triphosphate beta-1) and HSP (heat shock protein from mitochondrial membrane), an energetic index has been calculated and proposed to express tumoral metabolic status and prediction marker of tumoral aggressiveness (Guezva 2004).

A strong relationship has been found between high glycolytic phenotype and tumoral aggressiveness. Indeed, several facts indicate that, either FDG captation or Lactate levels determined by spectro MRI exams, constitute negative prediction factors in tumoral behavior [8, 9, 10]. It was stressed that Warburg effect might represent an adaptive mechanism for tumoral cells to overcome increased hypoxia rates and insufficient vascular supply. Consequently, targeting tumoral metabolism and, more specifically, glycolysis is considered a promising tool in anticancer therapy. Prior to deciding which anti-metabolite therapy would be the most productive, a thorough appreciation of energetic metabolism should be undertaken in order to confirm or infirm characteristics like: enhanced glycolysis, glutaminolysis [4]. However, there is a growing awareness that glycolytic phenotypes vary significantly, as many tumors cannot be visualized by FDG PET scans. Also, it has been suggested that nutrients other than glucose might be utilized as main energetic suppliers: glutamine and even lactate [5, 6, 7]. Few studies have addressed these issues or tried to explain these metabolic differences in stem cells derived from gliomas.

Stem cells that derive from tumors being denominated as cancer stem cells are seldomly regarded as therapy resistant factors and reflect the most aggressive part of the tumor. In our study, several lines of stem cells derived from different gliomas but appreciated to have the same WHO III malignancy degree have been tested for their susceptibility to glucose and glutamine withdrawal. Comparing different cell lines obtained from adult and child glioma and also normal neural stem cell lines, showed that the NADH production was constantly inferior for cancer stem cells with the highest proliferative index compared to those with the lowest ones.

Due to these results, the assumption that cancer cells are driven by high energy demanding processes and/or glycolysis upregulation results in more ATP (adenosine triphosphate) production than in normal cells has not been validated. Several studies capitalized on NMR spectroscopy (nuclear magnetic resonance spectroscopy) using 13C isotopomer for glucose labeling, drawing the conclusion that enhanced energetic and anabolic metabolism is driven by glycolysis [3]. Because glycolysis produces ATP less efficiently than aerobic respiration, tumor cells must compensate by having a much higher rate of glucose uptake than normal cells. In some malignant rapidly-growing tumors, glycolysis rates are up to 200 times higher than normal cells. AMPK (AMP-activated protein kinase) has emerged as a master regulator of cellular energy metabolism with dozens of downstream targets. The p53 modulator's main role is in protecting cells from DNA (Deoxyribonucleic acid) errors introduced into cell growth and division by diverse stress signals and acts by negatively regulating the IGF-1/mTOR pathways (insulin-like growth factor/mammalian target of rapamycin). IGF-1/mTOR hyper-activation is rarely encountered in tumoral cells, while p53 anti-oncogene is lost in almost 70% of tumors. The well known targets of p53 are the TSC2 (tuberous sclerosis), PTEN (Phosphatase and tensin homolog), and IGF genes- but also the A subunit of AMPK-AMPK β1 with direct implication in tumoral energy metabolism [5]. All these facts indicate that

energetic metabolism is rather inefficient in some types of tumoral cells, as for the same amount of glucose, less of NADH is produced. In our study, we have confirmed this assumption and found by direct NADH measurement that proliferative cellular indexes correlate indirectly with NADH production. The aberrant metabolism sustained by genetic abnormalities confers the obvious advantage by deviating energy resources to synthetic proliferative processes [6, 7, 8].

Further, we have tried to assess the influence of several anti-metabolites on NADH produced by either cancer or normal neural stem cells. We found that DCA, which acts as inhibitor of Pyruvate Dehydrogenase Kinase, accelerating oxidative phosphorilation, can enhance energetic metabolism in glioma stem cells, but has no effect on neural stem cells. This fact proves that despite genetic modification in tumoral cells, some functions, and more specifically oxidative phosphorilation functions, can be restored. It has been shown earlier that p53 loss results in a defect in oxygen consumption, which suggests a decreased function of mitochondrial respiration [6, 8]. Still, on the basis of glioma stem cell metabolism we verified a previously announced hypothesis of DCA enhancing properties on tumoral mitochondria. In the same study, we found that glucose was an obligatory fuel for several types of glioma, in our case for the stem cells derived from two child glioma and one adult glioma. In one case of stem cell line derived from adult glioma, the NADH production could be maintained solely by glutamine.

As it was expected, glycolysis inhibition by 2-Deoxy-D-Glucose resulted in marked NADH inhibition in cancer stem cells and only moderately for neural stem cells.

Conclusion

An aberrant energetic metabolism in cancer was confirmed on the basis of glioma stem cells compared to neural stem cells. Primarily, for an apparently similar degree of malignancy established anatomopathologically, energetic study showed significant differences in tumoral dependence on glucose and glutamine. Secondly, proliferative index correlated inversely with cellular energetic status.

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