Metabolic phenotypes of cells resistant to oxidative stress and apoptosis: common patterns and variations in glycolysis and glutaminolysis observed in 6 thymocyte cancer variants

N. W. Lutz[1,2], M. E. Tome[3], M. M. Briehl[3]

[1]Arizona Cancer Center, University of Arizona, Tucson, Arizona, United States, [2]Faculté de Médecine, Institut de Physique Biologique, Louis Pasteur University, Strasbourg, France, [3]Department of Pathology, University of Arizona, Tucson, Arizona, United States

INTRODUCTION

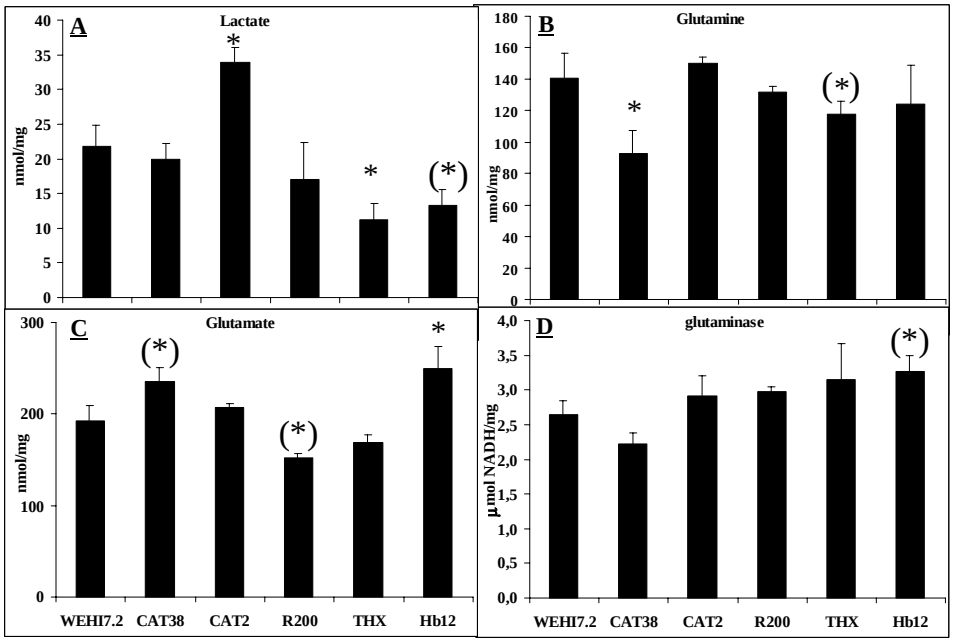
Since Otto Warburg's seminal discovery that tumor cells produce an increased amount of lactic acid from glucose when compared to normal cells, a large body of work has shown that alterations in glucose, glutamine and/or energy metabolism occurring in tumor cells provide these cells with growth and survival advantages in a tumor microenvironment where oxygen or specific nutrients may be scarce. Previous metabolomic studies based on ^{31}P MRS demonstrated that five steroid-resistant variants produced from the steroid-sensitive thymocyte cancer cell line, WEHI7.2, by either transfection or selection for increased resistance to oxidative stress, generally show an increased hexose/triose pool (mostly glycolytic intermediates) and a decreased bioenergetic status. We present here an extended analysis of glucose and glutamine metabolism associated with resistance to oxidative stress, based on ^{1}H and ^{31}P MRS determination of metabolic profiles as well as enzyme activity measurements. This investigation contributes to the elucidation of different strategies employed by five resistant thymocyte cancer cell lines to survive oxidative stress.

MATERIALS AND METHODS

Sample preparation Steroid-sensitive WEHI7.2 cells have been rendered resistant to oxidative stress and apoptosis by stable transfection with and overexpression of human bcl-2 (Hb12 variant), thioredoxin (THX variant) or catalase (CAT38 or CAT2 variants, expressing catalase 1.4 or 2-fold); or by selection for resistance to 200 μM H_2O_2 (200R variant) [1]. Typically, ca 6–8 × 10^8 cells were harvested by centrifugation, rinsed, and extracted with methanol/chloroform/water (1:1:1) as described previously [1]. The aqueous phase was lyophilized after evaporation of the organic solvents under a nitrogen stream, and prepared for ^{1}H and ^{31}P NMR spectroscopy as previously described [1,2]. Sample preparation protocols for the determination of glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH) and glutaminase activities have been adopted from [3], [4], and [5], respectively.

NMR spectroscopy and enzyme assays ^{1}H and proton-decoupled ^{31}P NMR spectra were obtained on a 11.7 T Bruker AVANCE DRX500 NMR spectrometer using a broadband probe for 5-mm tubes. For ^{1}H (^{31}P) spectra the acquisition time AQ was 3.28 (2.025) s, and the sweep width SW was 5 (2) kHz. Spectra were acquired at 4°C over 11 min (5-6 h) with a repetition time of TR = 5.3 (13) s [1,2]. Enzyme activities were determined according to refs. [3-5] as indicated in 'Sample preparation' above.

Statistics Data are reported as means of three or four independently collected samples for the NMR data. For all other measurements, a representative experiment from at least two replicates (three independent samples each) is shown. Fisher's PLSD test for multiple comparisons (StatView, SAS Institute, Cary, NC) was used to test for significant differences between individual steroid-resistant variants and the sensitive parental cell line, WEHI7.2. Plain asterisks (or asterisks in parentheses) indicate $p < 0.05$ (or $0.05 < p < 0.08$, i.e. borderline significance).



RESULTS

Although both catalase-transfected variants showed significantly increased levels of phosphorylated glycolytic intermediates [1] and hexokinase activity (not shown) when compared to steroid-sensitive WEHI7.2 cells, only one of them, CAT2, exhibited *increased* lactate (Fig.A) and LDH (not shown) levels. The other steroid-resistant variants showed unchanged or slightly decreased lactate levels. In contrast, glutamine levels were significantly *decreased* for CAT38 cells, but not for CAT2 or the other resistant variants (Fig.B). The slight glutamate (Fig.C) and aspartate (not shown) increase in CAT38 cells in the absence of glutaminase increase (Fig.D) suggests that glutaminase activity is not rate limiting in the glutaminolytic production of glutamate and aspartate from glutamine. In conjunction with previously reported amino acid patterns and increased use of mitochondria for ATP production in the steroid-resistant variants [2], our data suggest that increased glutaminolysis may be one of the metabolic processes distinguishing the steroid-resistant variants from the steroid-sensitive parental cell line.

DISCUSSION

The differences in glycolytic and glutaminolytic behavior between WEHI7.2, CAT38 and CAT2 cells clearly highlight the existence of a *non-monotonic* relationship between the degree of catalase overexpression (1.0, 1.4 and 2.0-fold, respectively) on the one hand, and glycolysis and glutaminolysis modulation on the other. Furthermore, the absence of a uniform trend for the levels of glycolytic and glutaminolytic end products and enzyme activities in sensitive vs. resistant cancerous thymocyte cells indicates that these variants may use different metabolic strategies to foster resistance to oxidative stress and steroid-induced apoptosis. Since increased lactic acid production is often associated with enhanced tumor invasion [6], the capability of cancers to grow in a hostile environment, by replacing less aggressive cells, may be closely related to mechanism(s) of resistance for cell lines such as CAT2. However, this lactogenic mechanism is unlikely to play a significant role in the other steroid-resistant variants investigated where lactate and LDH levels were relatively low. Enhanced glutaminolysis, which is an oxidative process, observed for resistant variants vs. parental WEHI7.2 cells indicates an increased ability to handle ROS production occurring during mitochondrial ATP production. Thus, oxidative-stress resistant cells may be better able to remove or tolerate ROS than sensitive cells. However, the metabolic details of the glutaminolytic pathways employed by the cells vary considerably between the variants. Overall, these results suggest that thymocyte cancer cells are able to use glycolytic and glutaminolytic strategies to foster resistance to steroid-induced apoptosis, and that the metabolic control of these processes varies in a complex manner as a function of the method used to render cells resistant to oxidative stress.

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