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Resistance exercise prevents impaired homocysteine metabolism and hepatic redox capacity in Walker-256 tumor-bearing male Wistar rats

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ABSTRACT

Objective: The aim of this study was to investigate changes in homocysteine (Hcy) metabolism and redox balance in response to exercise treatment in a tumor-bearing rat model.
Methods: Male Wistar rats were exposed, or not, to a resistance exercise program 6 wk before inoculation with Walker-256 tumor cells or vehicle. After application, rats maintained their routine for 12 d and were then sacrificed for plasma and liver analyses.
Results: Impaired Hcy metabolism was evident after 12 d of tumor cell inoculation as demonstrated by significantly increased ($P < 0.05$) plasma total homocysteine (tHcy) concentration (53%) and decreased plasma cysteine, methionine, and vitamin B_{12} concentrations. Decreased hepatic cystathionine β-synthase (CBS) and betaine-homocysteine *S*-methyltransferase mRNA levels were found in tumor-bearing rats but not in controls. Tumor inoculation also decreased levels of liver reduced glutathione (GSH) and increased hepatic oxidative stress compared with non-tumor controls. However, resistance exercise prevented the tumor-impaired transsulfuration pathway as demonstrated by the decreased plasma tHcy, hepatic CBS expression, and increased GSH in tumor-exercised versus tumor-sedentary rats. Remarkably, all measures of liver oxidative stress were suppressed by exercise training. Tumor weight was unchanged between groups.
Conclusion: Resistance exercise prevented tHcy accumulation and liver oxidative damage caused by Walker-256 tumor cell inoculation; the modulatory effects of resistance exercise on Hcy metabolism appear to be at the level of transsulfuration pathway.

Introduction

The importance of balancing systemic homocysteine (Hcy) levels is substantiated by its association with numerous diseases, and their pathologies [1]. Hcy is an amino acid that is formed exclusively by demethylating methionine during the cellular process of transmethylation [2]. Under normal circumstances, Hcy concentrations are precisely controlled by two pathways that either catabolize Hcy to cysteine, or methylate Hcy to reform methionine [3]. Hcy catabolism occurs by the two-step process of transsulfuration that is initiated by the enzyme cystathionine-β-synthase (CBS). CBS forms cystathionine by combining Hcy with the amino acid serine. The end product of the transsulfuration pathway is cysteine, which plays a key role in redox balance

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through the synthesis of glutathione (GSH), a major antioxidant [4]. In contrast, Hcy is also remethylated back to methionine via two pathways. Hcy remethylation either occurs via methionine synthase (MS), an enzyme that transfers a methyl group from 5-methyltetrahydrofolate, or via betaine-homocysteine *S*-methyltransferase (BHMT), an enzyme that transfers a methyl group from betaine [5,6]. Indeed, remethylation and transsulfuration balance both methyl and redox homeostasis (Fig. 1), and the apparent disruption of this balance is observed in several diseases.

Numerous studies indicate that cancer development causes perturbations in Hcy metabolism, which result in elevated plasma total Hcy concentrations and liver damage [6–8]. Furthermore, the observed accumulation of plasma Hcy in cancer patients appears to be a consequence of limited hepatic transsulfuration capacity. For example, malignant cells lack the capacity to remethylate Hcy to methionine [6], and thus proliferating tumor cells undergoing rapid transmethylation result in systemic Hcy accumulation. Moreover, the high methyl demand of tumor cells would likely inhibit Hcy elimination by transsulfuration in the liver [9]. A consequence of impaired transsulfuration capacity is reduced endogenous production of antioxidants such as GSH. Indeed, because Hcy is prone to oxidation itself and is associated with oxidative stress [10], Hcy accumulation may further disrupt redox status and contribute to tumor progression. However, little is known about the development of abnormal Hcy metabolism in response to tumor progression and pathogenesis.

Recently, exercise training has emerged as a means of preventing metabolic perturbations linked with carcinogenesis and tumor-induced cancer [11,12]. Indeed, exercise training was demonstrated to upregulate impaired Hcy metabolism in rats fed folate-restricted diets [13], as well as in healthy [14,15] and overweight humans [16]. However, it has not been determined whether exercise training modulates Hcy metabolism during cancer progression. The aim of this study was to investigate Hcy metabolism and redox balance in response to exercise treatment in a tumor-bearing rat model.

Methods

Animals and study design

In all, 38 male Wistar rats, weighing 252.4 ± 19.4 g (~8 wk old), were obtained from the Biological Sciences Center at the State University of Londrina. All procedures were approved by the Ethics Committee for Animal Use at the same institution, and were in accordance with the Guidelines of the COBEA (Brazilian College of experiments with animals). The rats were weighed every other day and were housed in collective cages on a 12-h light/dark cycle at a mean temperature of 22°C. Rats were randomly divided into the following groups: control (C, n = 9), tumor-bearing (T, n = 10), resistance exercised (E, n = 9), and tumor-bearing resistance exercised (TE, n = 10). The rats had free access to food (commercial diet Nuvilab®, Quimtia,

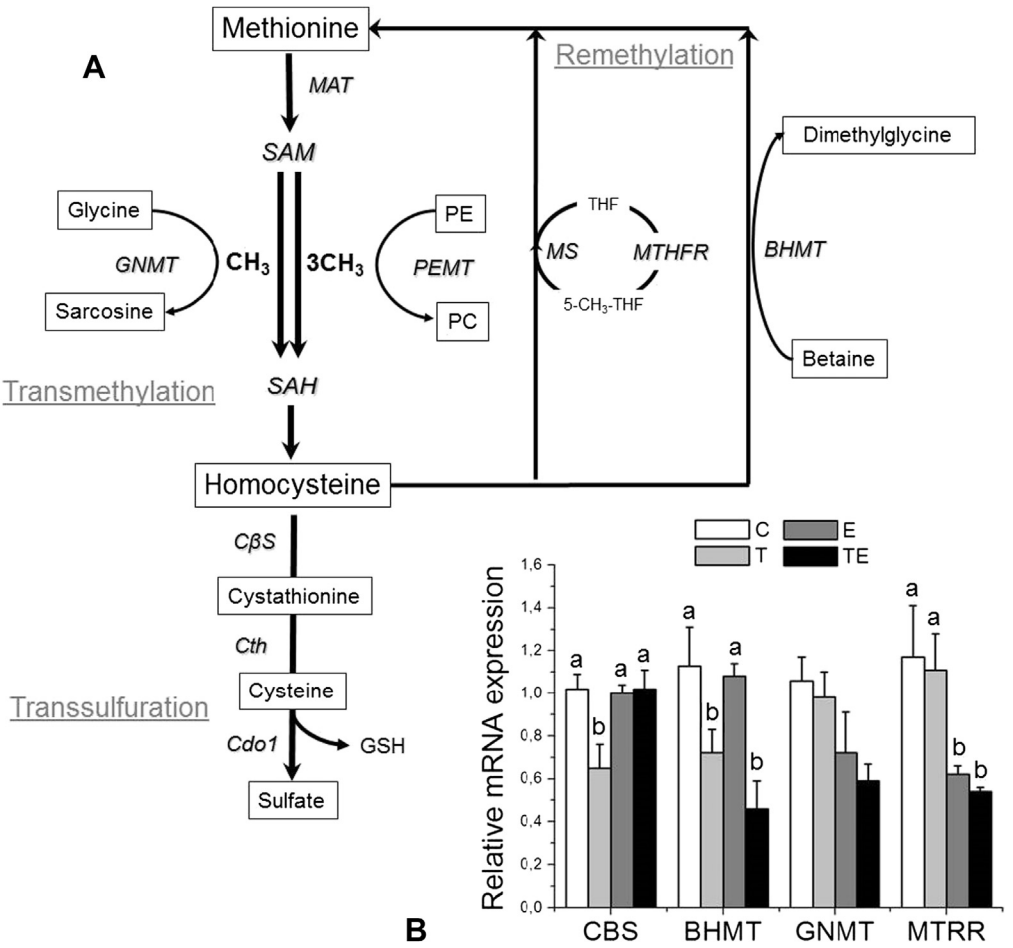

Fig. 1. Hcy metabolism. (A) Expression of key genes involved in Hcy metabolism in the control (C), tumor-bearing (T), exercised (E), and tumor-bearing exercised (TE) rats. (B) Data are mean ± SEM, n = 7. Means without a common letter differ among groups ($P < 0.05$). CBS, cystathionine-beta-synthase; BHMT, betaine-homocysteine S-methyltransferase; GNMT, glycine N-methyltransferase; Hcy, homocysteine; MTRR, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase.