The Glucose Dependent Transcription Factor ChREBP Contributes to Glucose-Dependent Anabolic Synthesis and Cell Proliferation

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Introduction

Many human tumors display high rates of aerobic glycolysis, *de novo* fatty acid synthesis and nucleotide biosynthesis (1). Previous findings suggest that increases in these processes are promoted by rapid glucose metabolism (2). Although these metabolic alterations might not be initiating events in oncogenesis, blocking tumor metabolism has been shown to be a useful strategy for slowing carcinogenesis (3). Thus, understanding the genes that are required to support anabolic metabolism in normal and tumor cells may provide new strategies for cancer therapy and prevention. The basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factor carbohydrate responsive element binding protein (ChREBP) is a critical mediator of glucose-dependent induction of glycolytic and lipogenic enzyme genes (4). Cellular levels of nutrients (such as glucose and fatty acids) regulate the level and activity of ChREBP in hepatocytes and adipocytes (5). ChREBP null mice show decreased glycolysis and lipogenesis as well as intolerance to dietary carbohydrate (6). Although the function of ChREBP in hepatocytes has been extensively investigated, little is known about the role of ChREBP in proliferating or transformed cells. In this study, the role of ChREBP in cancer cell proliferation and metabolism was investigated. ^{13}C and ^{14}C labeled glucose were used to trace primary and secondary metabolic pathways in normal and CHREBP knock-down human colon cancer cells.

Methods

HCT116 cells (human colon cancer) were cultured in Dulbecco's Modified Eagle's Medium (DMEM), which contained 25 mM glucose, 4 mM glutamine, 10 mM HEPES and 10% fetal bovine serum. HCT116 cells were transfected with either control or ChREBP siRNA (Invitrogen, Carlsbad, CA). The transfecting agent was Lipofectamine RNAiMax (Invitrogen); two different ChREBP siRNA's were compared. After transfection, cells were allowed to grow for 3 days in 10 cm dishes and subsequently labeled in medium containing 7 mM ^{13}C glucose for 5 hours. [1,6-$^{13}C_2$]glucose was used to analyze the TCA cycle and [2-^{13}C]glucose was used to examine the pyruvate carboxylase flux (7). Cells were extracted with perchloric acid, neutralized with KOH, lyophilized, and analyzed in D_2O. The total number of cells used for each extract ranged from 5×10^6 to 1×10^7. Experiments were performed in duplicate. ChREBP knockdown was confirmed with a Western blot; a rabbit polyclonal antibody raised against part of the ChREBP protein was used (Novus Biologicals, Littleton, CO)

NMR spectra were acquired with a 400 MHz system (Varian Inc. Palo Alto, CA). A 5-mm broad band probe was used to acquire NOE-enhanced ^{13}C spectra. Acquisition parameters were: PA = 60°, bilevel WALTZ16 decoupling, TR = 3000 ms, 32K points and spectral width = 30000 Hz. Free induction decays were zero filled to 65536 points and apodized with exponential multiplication (1.5 Hz). Resonances areas were determined with Nuts NMR (AcornNMR, Livermore, CA).

Glucose and lactate levels were determined with a bench-top analyzer (Nova, Waltham, MA) and oxygen consumption was determined with trypsinized cells in a stirred micro chamber with a Clarke electrode at 37 C. Biosynthesis of lipids and nucleic acids were determined with [U-$^{14}C_6$]glucose (8).

Results and Discussion

Knock down of ChREBP with either of the two siRNA's resulted in reduced glucose consumption (see bar graph below) and lactate formation (not shown). It also resulted in increased oxygen consumption (see bar graph). ^{14}C results demonstrated that ChREBP knock-down reduced the flux from glucose to nucleic acids through the pentose phosphate pathway as detected in RNA extracts (data not shown). Incorporation of ^{14}C from glucose into lipids was also reduced (data not shown).

In the NMR spectra, many resonances were observed for the control HTC116 cells, including those for C-3 of alanine and lactate, the 3 central carbons of glutamate and glutamine, C-4 glutathione, glycolytic intermediates and the natural abundance ^{13}C in HEPES. The high ratio of labeling in C-4 to C-3 and C-2 is similar to that reported previously with other cancer cell types with [1,6-$^{13}C_2$]glucose (9). This is believed to be the result of a high pyruvate dehydrogenase flux relative to pyruvate carboxylase flux and a low TCA cycle flux relative to unenriched anaplerotic flux (i.e., from unlabeled amino acids into intermediates such as α-ketoglutarate, oxaloacetate and succinyl-CoA). With ChREBP knock down, marked reductions were observed for lactate, alanine and the glycolytic intermediates, consistent with a reduced glycolytic flux. Also, glutathione levels were reduced to below the detectable limit. The ratio of label in C-2:C-4 glutamate increased from 0.23 ± 0.04 to 0.37 ± 0.07 ($p < 0.05$), which suggests an increased TCA cycle flux, relative to anaplerotic fluxes. To examine if the increase in C-2 glutamate labeling was due to an increase in pyruvate carboxylase flux, cells were labeled with [2-^{13}C]glucose (7). With PDH flux, label will be predominately transferred to C-5 of glutamate, whereas with PC flux, label will be predominately transferred to C-2 of oxaloacetate and eventually, glutamate C-2 and C-3 (7). ^{13}C NMR analysis revealed that for the control cells, only a slight enrichment was observed in glutamate C-3 and a much stronger enrichment was observed for glutamate C-5. After correction for T1 relaxation, the PDH flux was estimated to be more than 10 times the PC flux. ChREBP knock-down did not significantly change this ratio. Therefore, the increase in glutamate C-2 relative to glutamate C-4 appears to be due to an increase in the TCA cycle flux.

Conclusions

ChREBP knockdown had marked effects on HCT116 colon cancer cell metabolism. It reduced the flux of glycolysis and a number of biosynthetic pathways and converted cells to a more quiescent phenotype. The results suggest that ChREBP may be an important target in cancer therapy.
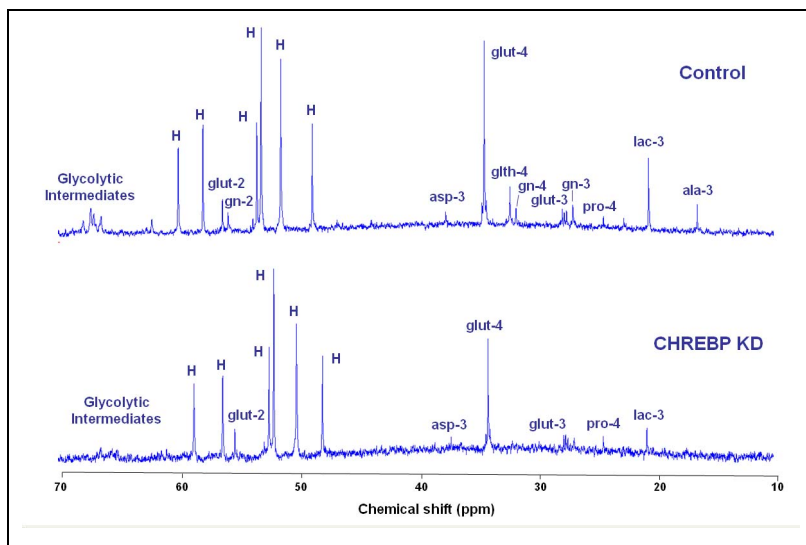
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