HIGHLY EXPRESSED UCP2 ENHANCES GLUCOSE METABOLISM THROUGH WNT SIGNALING

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ABSTRACT

Uncoupling protein 2 (UPC2) is an inner mitochondrial membrane protein that acts as an anion/ion transporter. UPC2 is often highly expressed and plays two roles in cancer: functioning as a protective mechanism against oxidative stress and chemotherapy; and promoting cancer metabolism for cancer growth and progression. UCP2 overexpression also plays a key role in cellular metabolism during cell transformation. JB6 is a cell line of mouse epidermal cells that were used for cell transformation. Upregulation of UCP2 within these cells resulted in enhanced glycolysis. To identify the underlying mechanism, glucose PCR array analysis was performed using mRNA extracted from control and UCP2 overexpressed JB6 cells. Our data found that 18 genes were upregulated and 6 were downregulated. Quite a few of these genes were found to belong to the Wnt signaling, which include the Frizzled, Wnt, and DVL gene families. These data suggest UCP2 may regulate glucose metabolisms through Wnt signaling.

OBJECTIVES

- Determine how highly expressed UCP2 regulates glucose metabolism

METHODS

Cell Culture:

JB6 is a murine cell line of epidermal cells. They are a transformation cell line used to study tumor promotion. The cell line was purchased from American Type Culture Collection (ATCC). UCP2 overexpression and control PCMV6 vectors were purchased from OriGene and transfected into the JB6 cell line and produced stable clones.

JB6 cells were seeded in p100 dishes and when cells became confluent, washed with cold phosphate buffered saline (PBS) and harvested for mRNA isolation.[1 2]

METHODS (CONT.)

PCR array:

RNA was isolated from the cells using Qiagen's RNeasy Micro Kit flowing the manufacturer's procedures. cDNA synthesis was conducted using Qiagen's RT² First Strand Kit. RT² Profiler Mouse Glucose PCR Array from Qiagen was used to quantity gene expression of the sample. Prepared cDNA from the reverse transcription was used in the array in combination with RT² SYBR® Green qPCR Mastermix from Qiagen. The PCR array was conducted using a Bio-Rad CFX384 Touch Real-Time PCR system.

Data analysis:

The resulting analysis produced C_t values using the delta C_T method. Delta C_T was calculated from the gene of interest (GOI) and the average of reference genes (HKG). The resulting value was used to calculate the delta-delta C_T from the delta C_T (test group) – delta C_T (control group). The fold change was calculated using the formula $2^{\wedge(-\text{delta delta CT})}$. The C_T cut off was set to 35. There was a two-fold regulation threshold.

RESULTS

The overexpression of UCP2 resulted in twenty-four genes being up or down regulated. Eighteen genes were upregulated (Table 1.) and six were downregulated (Table 2).

Seven Wnt family genes were identified in the array. PITX2 has a positive correlation to WNT3A. FGF4 may be regulated by Wnt. Conversely, CTNNBIP1 is a negative regulator of Wnt. Genes from the Frizzled and Lipoprotien families were identified in the array, these genes are receptors for Wnt signaling molecules. The Rho family of genes and pathway are interconnected with the Wnt pathway and play a significant role in cancer signaling cascades. The DVL family of genes interact with Rho family as well as DAB2. KREMEN1 works to block Wnt signaling.

RESULTS (CONT.)

Table 1. Upregulated Genes from RT² Profiler PCR Array and fold regulation.

Gene Symbol	Fold regulation
CTNNBIP1	2.04
DAB2	2.19
DVL1	2.62
DVL2	2.65
EP300	2.03
FGF4	2.13
FZD1	11.74
FZD9	2.17
KREMEN1	2.66
LRP6	2.72
MMP7	2.18
RHOA	3.05
WNT1	2.27
WNT2B	2
WNT3	2.26
WNT7B	3.23
PRMT6	3.31
RPLP0	2.02

Table 2. Downregulated Genes from RT² Profiler PCR Array and fold regulation.

Gene Symbol	Fold regulation
CTBP1	-2.18
FZD3	-2.07
PITX2	-3.54
WNT10A	-2.09
WNT2B	-2.01
WNT7A	-3.13

DISCUSSION

Twenty of the genes identified play a role in the Wnt signaling pathway. Wnt signaling seems to increase glucose metabolism by increasing the expression and activity of several genes and enzymes such as Adenosine triphosphate/Adenosine diphosphate (ATP/ADP), phosphofructokinase (PFK), and glucose-6 phosphate dehydrogenase. Metabolism of glucose is necessary to supply energy to cells in the body. Cancer cells typically show increased uptake of glucose and high aerobic glycolysis. Targeting glucose metabolism is difficult to target in cancer therapy. [3]

The data suggests that UCP2 overexpression may regulate glucose metabolism through Wnt signaling. The overexpression of UCP2 resulted in a change in expression of several Wnt signaling pathway genes. Wnt signaling seems to increase glucose metabolism in within the cell. Wnt signaling may be a potential target to regulate glucose expression in cancer therapy in the future through use of UCP2 expression.

References

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