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Oxidative Stress and Metabolic Reprogramming in Cr(VI) Carcinogenesis

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Abstract

Cr(VI)-containing compounds are well-established lung carcinogens. Chronic exposure of the normal human epithelial cells is able to induce malignant cell transformation, the first stage of metal carcinogenesis. These Cr(VI)-transformed cells exhibit increased level of antioxidants, reduced capacity of generating reactive oxygen species (ROS), and development of apoptosis resistance, promoting tumorigenesis of Cr(VI)-transformed cells, the second stage of metal carcinogenesis. The mechanism of Cr(VI) induced carcinogenesis is still under investigation. Recent studies indicate that ROS play a positive role in the first stage while a negative role in the second stage. Transformed cells adapt metabolism to support tumor initiation and progression. Altered metabolic activities directly participate in the process of cell transformation or support a large requirement for nucleotides, amino acids, and lipids for tumor growth. In malignantly Cr(VI)-transformed cells, mitochondrial oxidative phosphorylation is defective, and pentose phosphate pathway, glycolysis, and glutaminolysis are upregulated. These metabolic reprogramming supports rapid cell proliferation and contributes to tumorigenesis of Cr(VI)-transformed cells. This article summarizes the current progress in the studies of metabolic reprogramming and Cr(VI) carcinogenesis with emphasis on the metabolic enzymes and oxidative stress related major oncogenic pathways.

Keywords

Oxidative stress; Chromium (VI); Carcinogenesis; metabolic reprogramming

Introduction

When normal cells are converted to malignant transformed cells and progress to cancer, the metabolism is altered. In contrast to the normal differentiated cells which rely mainly on

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Declaration of interest

There are no conflicts of interest to declare.

mitochondrial oxidative phosphorylation (OXPHOS) for generation of needed energy, cancer cells depend on anaerobic glycolysis, a phenomenon called “the Warburg effect” for energy. This altered cellular metabolism, also called metabolic reprogramming, is recognized as one of the cancer phenotypes. Accumulative evidence reveals that various oncogenic pathways are involved in the metabolic regulation. Expression of glucose transporters and glycolytic enzymes are increased in numerous cancers and may contribute to tumor progression [1, 2]. It has been reported that oncogenes and tumor suppressor genes, such as hypoxia inducible factor 1 (HIF-1) [3], c-Myc [4–6], p53 [7,8], and PI3K/Akt [9], directly promote metabolism of glucose and glutamine.

Chromate (Cr(VI)) compounds, widely used in industry, have been shown to be toxic and carcinogenic on humans [10–13]. Cr(VI) is structurally similar to sulfate and phosphate anions; therefore, it readily enters into the cells via non-specific anion transporters [14]. Once inside the cells, Cr(VI) undergoes a series of metabolic reductions and forms intermediate Cr species, including Cr(V) and Cr(IV), and is finally reduced to Cr(III) [14, 15]. In the Cr(VI) reduction process, reactive oxygen species (ROS) are produced, resulting in oxidative DNA damage. The intermediates Cr(V), Cr(IV), and the final product Cr(III) are very reactive, causing Cr-DNA adducts and genomic alterations. Epidemiological studies have shown that occupational exposure to Cr(VI) is associated with a high rate of lung cancer in workers employed in these industries [10–13]. Environmental Cr(VI) exposure is also a public health concern and is associated with long-term carcinogenic effects of the lung [12, 16]. The mechanisms of Cr(VI) carcinogenesis have not yet been fully understood, it generally believed that ROS are important in inducing malignant cell transformation, the first stage of metal carcinogenesis [3–5, 17*]. ROS can be involved in various carcinogenic processes [17*]. Recent studies from our laboratories have shown that once cells are malignantly transformed, the capacity of those transformed cells to generate ROS is sharply reduced, contributing to the development of apoptosis resistant and subsequent tumorigenesis [18, 19*]. Thus, the decreased ROS generation in Cr(VI)-transformed cells is oncogenic in promoting tumorigenesis, the second stage of metal carcinogenesis. The oncogenic role of ROS in the first stage of Cr(VI) carcinogenesis (malignant cell transformation) and anti-oncogenic role in the second stage (tumorigenesis) reflects the metabolic reprogramming during the change from normal cells to malignantly transformed cells. Although this reprogramming may play an important role in the mechanism of metal carcinogenesis in general and Cr(VI) carcinogenesis in particular, its underlying mechanism remains to be investigated. This article provides an outline on progress and future perspectives in oxidative stress and metabolic reprogramming in Cr(VI) carcinogenesis.

Glycolysis

Glucose homeostasis is controlled by glycolysis/OXPHOS and gluconeogenesis pathway. Glycolysis is the enzymatic conversion of glucose into lactate, which produces 2 ATP per glucose molecule. In the presence of oxygen, normal cells primarily adopt mitochondrial OXPHOS to produce 36 ATP per glucose molecule. Cancer cells favor aerobic glycolysis over OXPHOS to meet their energy demand, suggesting that cancer cells are adapted to survive and proliferate in the absence of mitochondrial ATP production. Mitochondria play a major role as energy suppliers and ROS regulators. Although various mechanisms of

carcinogenesis induced by Cr(VI) have been demonstrated, it is generally believed that Cr(VI)-induced oxidative stress is important in converting normal cells to malignantly transformed cells. It has been reported that Cr(VI) suppressed all five mitochondrial complexes involved in OXPHOS in a variety of model systems with higher potency of complexes I, II, and V than complexes III and IV. Our recent study has observed that in Cr(VI)-transformed cells mitochondrial ATP production was reduced and non-mitochondrial oxygen consumption was increased, indicating the defect of mitochondrial ATP production [20**]. The results from RNA sequencing analysis show that levels of various enzymes involved in all five complexes were reduced in Cr(VI)-transformed cells compared to passage-matched normal cells (Table 2), demonstrating that Cr(VI)-transformed cells are defective in mitochondrial ATP production.

Aerobic glycolysis, maximizing ATP production, does not require an increase in mitochondrial capacity [21]. Cr(VI)-transformed cells generated more lactate without significant changes in glucose uptake and ATP production, indicating a switch from mitochondrial respiration to glycolysis [20**]. The results from Table 1 show that several glycolysis enzymes including ADP-specific glucokinase (ADPGK), enolase 1 (ENO1), hexokinase (HK2), phosphoglycerate kinase (PGK1), dihydrolipoamide S-acetyltransferase (DLAT), pyruvate dehydrogenase E1 (PDHA1), glucose-6-phosphatase 3 (G6PC3), pyruvate kinase (PKM), aldolase A (ALDOA), and phosphofructokinase (PFKM) were upregulated in Cr(VI)-transformed cells, indicating that Cr(VI)-transformed cells utilize glycolysis for energy production under defective mitochondrial function. It should be noticed that many metabolic enzymes are regulated through allostery and/or post-translation, such as pyruvate dehydrogenase, a complex with multiple subunits and cofactors, whose activity is regulated by phosphorylation/dephosphorylation. Thus, characterization of metabolic flux together with transcriptomics is more appropriate to evaluate the metabolic changes upon Cr(VI) exposure. Cancer stem cells (CSCs) or cancer-initiating cells, a small subset of malignant cells that exhibit high capacity of self-renewal and differentiation, have been reported to utilize aerobic glycolysis for biosynthesis and energy requirement [22]. About 1% of Cr(VI)-transformed cells have been identified as CSCs. These CSCs are metabolic inactive as evidenced by dramatic reductions of glucose uptake, lactate production, and ATP content [20**]. These small population of CSCs may be the driving force for the increased glycolysis of Cr(VI)-transformed cells.

Energy metabolism is a balanced mechanism controlled by catabolic (glycolysis and oxidative phosphorylation) and anabolic (gluconeogenesis) reactions. Fructose-1, 6-bisphosphatase (FBP1), a rate limiting enzyme in gluconeogenesis, catalyzes the hydrolysis of fructose-1, 6-bisphosphate to fructose 6-phosphate and inorganic phosphate. It has been reported that loss of FBP1 is correlated with advanced stage and poor prognosis of cancer [23, 24]. Inhibition of FBP1 increased glucose uptake and lactate secretion in HK-2 human renal cells and in consistent, forced expression of FBP1 reduced glucose uptake, lactate secretion, and glucose-derived TCA cycle intermediates in renal carcinoma RCC10 cells [25*]. In CSCs low level of FBP1 is beneficial for cancer cell growth due to (a) inductions of superiority of glycolysis and increased glucose uptake, facilitating the production of glycolysis intermediates and the energy supply during hypoxia and (b) inhibition of ROS generation induced by mitochondrial complex 1, protecting cells from oxidative stress [26].

In Cr(VI)-transformed cells FBP1 level is low compared to that in passage-matched normal cells and FBP1 is lost in CSCs [20**]. Ectopic expression of FBP1 in CSCs reduced glucose uptake, lactate production, and glycolysis [20**], indicating that FBP1 plays an important role in glucose metabolism.

HIF-1 α is important in angiogenesis and in cancer development [27–29]. Its level is elevated in more than half of human cancers and their metastases [30–35]. The occurrence of Warburg effect indicates the activation of oncogenic signaling, such as hypoxia inducible factor (HIF)-1 α , resulting in promotion of glucose uptake and anabolic metabolism [21]. This transcription factor upregulates many glycolytic enzymes, in which their gene promoters contain consensus binding motif 5'-(C/G/T)ACGTGC(G/T)-3' of HIF-1 α . HIF-1 α protein is rapidly degraded at normoxia via pVHL-mediated ubiquitin-proteasome pathway, whereas hypoxia blocks degradation of HIF-1 α protein, leading to its accumulation [36]. Stabilization of HIF-1 α modulates metabolic adaptation to low molecular oxygen levels through increase of cellular glycolysis [37]. HIF-1 α upregulates glucose transporters and glycolytic enzymes [38*]. HIF1 α also upregulates pyruvate dehydrogenase kinases (PDKs), the enzymes control entry of glucose-derived pyruvate into tricarboxylic acid (TCA) cycle [39, 40]. HIF-1 α was activated in Cr(VI)-repeatedly exposed cells [41] or in Cr(VI)-transformed cells [42]. HIF-1 α is able to bind to five glycolytic enzymes including phosphofructokinase (PFK), aldolase (ALDA), phosphoglycerate kinase 1 (PGK1), enolase 1 (ENO1), pyruvate kinase (PKM), and lactate dehydrogenase (LDHA) [43]. The results from Table 1 show that these five enzymes are upregulated in Cr(VI)-transformed cells. It is very likely that HIF-1 α directly binds to the promoters of these glycolytic enzymes. FBP1 binds to HIF-1 α inhibitory domain, blocking its induction of glycolysis [25*]. Thus, reduced FBP1 level in Cr(VI)-transformed cells may induce glycolysis through decreased binding to HIF1 α .

Phosphorylation on metabolic enzymes also contributes to aerobic glycolysis [44]. PI3K/Akt phosphorylates hexokinase and PFK-2 [45] and promotes GLUT expression and plasma membrane localization [46], suggesting that activation of PI3K/Akt pathway promotes the Warburg effect by stimulating glucose uptake and further catabolism by glycolysis. It has been demonstrated that PI3K/Akt/p38 MAPK is responsible for HIF-1 α activation in Cr(VI)-transformed cells [41], indicating involvement of PI3K/Akt/p38 in the upregulation of glycolysis of Cr(VI)-transformed cells.

Pentose phosphate pathway (PPP)

Pentose phosphate pathway (PPP), a classic metabolic pathway, consists of oxidative and non-oxidative branches. The oxidative PPP converts glucose-6-phosphate (G6P), a glycolytic intermediate, into ribulose-5-phosphate and generates NADPH, which is used for glutathione production, detoxification, and biosynthesis of lipids. The non-oxidative branch involves reversible carbon-exchanging reactions with the final products as fructose-6-phosphate and glyceraldehyde-3-phosphate, which participate in glycolysis and downstream metabolic pathways [47*]. PPP is upregulated in many types of tumors [47*, 48]. The activities of glucose-6-phosphate dehydrogenase (G6PD) and transketolase (TKT), key PPP enzymes, were increased in cancer cells [49, 50]. An early study indicated that short-term

exposure of human erythrocytes to Cr(VI) did not exhibit effect on any of the three PPP enzymes, glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (PGD), and transketolase (TKT) [51]. We have performed transcriptomics by RNA sequencing analysis. The results show that in Cr(VI)-transformed cells expressions of several PPP enzymes including phosphorybosyl pyrophosphate synthase 1 & 2 (PRPS1/2), G6PD, ribulose 5-phosphate 3-epimerase (RPE), transaldolase (TALDO1), PGD, ribose 5-phosphate isomerase (RPIA), aldolase A (ALDOA), and TKT were elevated compared to passage-matched normal cells (Table 1), indicating that PPP is upregulated in Cr(VI)-transformed cells.

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a key transcription factor that regulates antioxidant proteins to neutralize ROS and to restore cellular redox balance [52*, 53]. This transcript factor plays dual roles in carcinogenesis. In normal cells, activation of inducible Nrf2 decreases malignant cell transformation via decrease of oxidative stress [54**–56]. Conversely, in cancer cells constitutively activated Nrf2 exerts oncogenic effects by protecting these cells from oxidative stress and chemotherapeutics [57*, 58]. Constitutive activation of Nrf2 has been identified in several types of human cancer cell lines and tumors [54**, 59–61]. Cancers with high Nrf2 level are associated with poor prognosis [62, 63], resistance to therapeutics, and rapid proliferation [64, 65]. In addition to its role in regulation of oxidative stress, Nrf2 is also involved in the anabolic metabolism [65, 66**]. Nrf2 directly activates six genes involved in the PPP and nicotinamide adenine dinucleotide phosphate (NADPH) production pathway, including G6PD, PGD, TKT, TALDO1, and malicenzyme1 (ME1), through binding of this transcription factor to antioxidant response elements (AREs) of these gene promoters [66**]. Using [U-¹³C₆] tracer assay, it has been demonstrated that Nrf2 is required for purine nucleotide synthesis [66**]. During metabolic reprogramming Nrf2 redirects glucose and glutamine into anabolic pathways, protecting cancer cells from oxidative damage [66**]. Activation of Nrf2 increases glucose uptake through the PPP, subsequently producing NADPH [66**–68**]. Our studies have reported that Nrf2 is constitutively activated in Cr(VI)-transformed cells and its inhibition suppresses tumorigenesis of these transformed cells [19]. Whether Nrf2 regulates PPP remains to be investigated in Cr(VI)-transformed cells. Our unpublished results indicate that Nrf2 positively regulates G6PD, PGD, TKT, and TALDO1 through direct binding to the AREs of these gene promoters, resulting in upregulation of PPP.

Glutaminolysis

Along with increased aerobic glycolysis, increased glutaminolysis is recognized as a key feature of the metabolic profile of cancer cells [69]. In addition to glycolysis, many tumors also depend on glutaminolysis to fuel their cellular bioenergetics and metabolism. Glutaminolysis catabolizes glutamine to downstream metabolites such as glutamate and α -ketoglutarate, important intermediates to fuel TCA cycle of tumors. Similar to glycolysis, glutaminolysis supplies cancer cells with both ATP and crucial precursors for continuous biosynthesis and accelerated proliferation [70, 71]. Table 1 shows that levels of three glutaminolytic enzymes including glutaminase (GLS), aspartate aminotransferase 2 (GOT2), and glutamine fructose-6-phosphate transaminase 1 (GFPT1) were elevated in Cr(VI)-transformed cells, suggesting upregulation of glutaminolysis.

Nrf2 increases glutamine consumption through enhancing glutaminolysis and glutathione synthesis. Nrf2 indirectly activates transcription factor 4 (ATF4), which regulates serine/ glycine biosynthesis enzymes, supplying the substrates for glutathione and nucleotide production [72]. Nrf2 promotes glutathione synthesis from glutamine [37]. Nrf2 induces glutamate cysteine ligase (GCL), a key enzyme for glutathione synthesis, by directly activating the GCL encoding genes [73]. Nrf2 also increases the supply of cysteine by direct activation of the gene encoding cysteine transporter SLC7A11 [74]. Nrf2 is constitutively activated in Cr(VI)-transformed cells. The mechanism of Nrf2 in regulation of glutaminolysis in Cr(VI)-transformed cells has not yet been reported. In consideration of the findings from Table 1, it is very possible that Nrf2 targets GLS, which metabolizes glutamine to glutamate, providing a key nitrogen donor and carbon supply for the TCA cycle of Cr(VI)-transformed cells.

Conclusions and future perspectives

Metabolic reprogramming, a major hallmark of cancer, is characterized by upregulations of glycolysis, glutaminolysis, lipid metabolism, and pentose phosphate pathway. The metabolic reprogramming provides energy and metabolites to support rapid growth and proliferation of cancer cells. Chronic exposure of the cells to Cr(VI) causes malignant transformation. Similar to other cancer cells, these Cr(VI)-transformed cells have increased need for nutrients, energy, and biosynthetic activities to produce all macromolecular components during each passage through cell cycle. Cr(VI)-induced tumorigenesis is a chronic process. Among all studies related to bioenergetic phenotype induced by Cr(VI), most of studies focused on the short-term exposure. Only a few studies investigated the metabolic activities after long-term exposure to Cr(VI). For several decades, there has been a concentrated effort to identify the mechanisms of Cr(VI) carcinogenesis. However, little has been done to determine how changes of genes involved in glucose and glutamine metabolism contribute to Cr(VI) carcinogenesis. Cr(VI) exposure interferes with metabolic transduction pathways through different levels, including gene expression, intracellular protein levels, and protein function. We speculate that oxidative stress plays an important role in these processes. Chronic exposure of the cells to Cr(VI) causes ROS generation, leading to malignant cell transformation. Cr(VI)-transformed cells exhibit reduced capacity to generate ROS and elevated levels of antioxidant enzymes, contributing to development of apoptosis resistance and tumor growth. In Cr(VI)-transformed cells, PI3K/Akt/p38 signaling is activated, resulting in upregulation of glycolysis. Constitutive activation of Nrf2 in Cr(VI)-transformed cells enhances the PPP and NADPH generation, promoting cell proliferation. A representative scheme of possible mechanisms of metabolic reprogramming in Cr(VI) carcinogenesis is summarized in Figure 1. Many questions remain to be answered concerning the metabolic reprogramming in mechanism of carcinogenesis induced by Cr(VI) as well as other carcinogenic metals, such as arsenic, nickel, and cadmium. (a) Because Cr(VI) alters certain key proteins, which are important in metabolic reprogramming, it is important to understand whether certain metabolism-related pathways are the cause or the results of Cr(VI)-induced cells transformation and tumorigenesis. (b) It is also important to investigate whether and how metabolic reprogramming changes the cell process beyond energy metabolism, energy storage, and production of precursors for

biosynthesis, which are major functions supporting malignancy and tumor growth. This study should cover the role of metabolic reprogramming in a broad area of Cr(VI) carcinogenesis, including cell cycle regulation, autophagy, malignant cell transformation, ROS generation, mitochondrial injury, apoptosis resistance, angiogenesis, migration, and invasion. (c) Many metabolic enzymes are regulated by allosteric binding or post-translational modification; for that reason, future studies should characterize metabolic fluxes to precisely evaluate the metabolic changes induced by Cr(VI). (d) It is also important to investigate whether interruption or alteration of metabolic reprogramming reduces Cr(VI)-induced malignant cells transformation and tumorigenesis. This study will lead to a new understanding on the mechanism of Cr(VI) carcinogenesis and its possible mechanism-based prevention.

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Abbreviations

ACO1	Aconiatse
ADPGK	ADP-specific glucokinase
ALDOA	Aldolase A
ARE	Antioxidant response element
ATF4	Transcription factor 4
CSCs	Cancer stem cells
COX	Cytochrome C oxidase
CS	Citrate synthase
DLAT	Dihydrolipoamide S-acetyltransferase
DLST	Dihydrolipoamide S-succinyltransferase
ENO1	Enolase 1
FBP1	Fructose-1,6-biosphosphate
FH	Fumarase
G6PC3	Glucose-6-phosphatase 3
G6PD	Glucose 6-phosphate dehydrogenase
GCL	Glutamate cysteine ligase
GFPT1	Glutamine-fructose-6-phosphate transaminase 1
GLS	Glutaminase

GOT2	Aspartate aminotransferase, mitochondrial
GPI	Glucose-6-phosphate isomerase
HIF	Hypoxia inducible factor
HK2	Hexokinase
MDH2	Malate dehydrogenase
ME1	Malicenzyme 1
NDUF	NADH dehydrogenase
NADPH:	Nicotinamide adenine dinucleotide phosphate
Nrf2	Nuclear factor erythroid-(derived 2)-like 2
OGDH	Oxoglutarate dehydrogenase
OXPHOS	oxidative phosphorylation
PDHA1	Pyruvate dehydrogenase E1
PFKM	Phosphofructokinase
PGD	6-phosphogluconate dehydrogenase
PGK1	Phosphoglycerate kinase 1
PKM	Pyruvate kinase
PPP	Pentose phosphate pathway
PRPS	Phosphorybosyl pyrophosphate synthetase
RPE	Ribulose 5-phosphate 3-epimerase
RPIA	Ribose 5-phosphate isomerase
ROS	Reactive oxygen species
SDHA	Succinate dehydrogenase
SUCLG1	Succinyl-CoA synthase
TALDO1	Transaldolase
TKT	Transketolase
UQCR	Ubiquinol cytochrome C reductase;

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(* of special interest and ** of outstanding interest)

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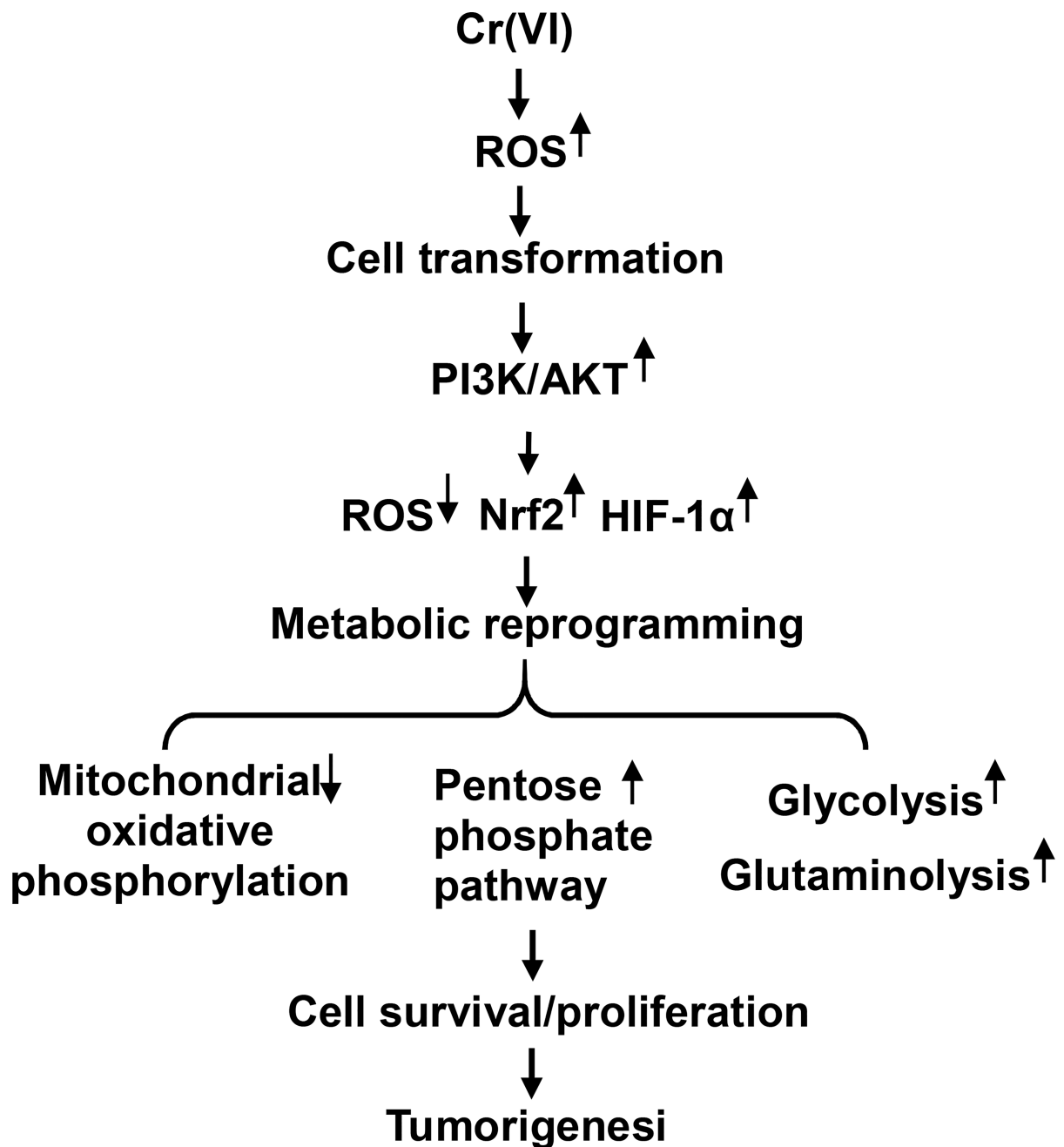


Figure 1. Representative scheme of metabolic reprogramming in Cr(VI) carcinogenesis
 Chronic exposure of cells to Cr(VI) causes ROS generation which is responsible for malignant cell transformation. Once the cells are malignant transformed, those cells exhibit activated PI3K/Akt, reduced ROS generation, and elevated levels of antioxidants and HIF-1 α , resulting in reduction of mitochondrial oxidative phosphorylation and upregulations of pentose phosphate pathway, glycolysis, and glutaminolysis, leading to tumorigenesis.

Table 1

Relative level of metabolic enzymes in Cr(VI)-transformed cells and passage-matched normal cells

Gene Symbol	Target pathway	BEAS-2B	BEAS-2B-Cr(VI)
ADPGK	Glycolysis	0.4 ± 0.6	6.7 ± 3.2
ALDOA	PPP/Glycolysis	6.8 ± 5.3	254.9 ± 57.0
DLAT	Glycolysis	14.7 ± 1.3	21.6 ± 2.6
ENO1	Glycolysis	1.5 ± 0.8	71.6 ± 0.9
G6PC3	Glycolysis	50.7 ± 4.1	97.3 ± 14.9
GPI	Glycolysis	19.1 ± 4.8	73.8 ± 8.9
HK2	Glycolysis	21.2 ± 4.7	32.1 ± 2.8
PDHA1	Glycolysis	48.9 ± 5.5	158.5 ± 11.7
PFKM	Glycolysis	29.5 ± 5.8	83.9 ± 3.6
PGK1	Glycolysis	107.5 ± 44.6	221.7 ± 68.5
PKM	Glycolysis	112.5 ± 86.5	261 ± 73.9
G6PD	PPP	29.2 ± 6.5	142.3 ± 50.2
PGD	PPP	6.8 ± 3.7	31.4 ± 5.9
PRPS1	PPP	59.4 ± 2.3	135.2 ± 8.1
PRPS2	PPP	27.2 ± 0.1	53.6 ± 5.9
RPE	PPP	28.1 ± 2.1	39.0 ± 4.7
RPIA	PPP	151.9 ± 39.7	324.3 ± 85.5
TALD01	PPP	28.8 ± 8.1	55.8 ± 7.7
TKT	PPP	151.9 ± 39.7	324.3 ± 85.5
GFPT1	Glutaminolysis	14.4 ± 1.7	26.8 ± 6.7
GLS	Glutaminolysis	19.3 ± 4.6	30.3 ± 10.4
GOT2	Glutaminolysis	99.4 ± 32.8	283.9 ± 36.7

Relative levels of metabolic enzymes involved in glycolysis, PPP, and glutaminolysis. Normal BEAS-2B cells (BEAS-2B) and Cr(VI)-transformed cells (BEAS-2B-Cr) were cultured in 10-cm dishes. After 90% of confluence, the cells were subjected for extraction and purification of RNA using RNAeasy mini kit. Whole transcriptome sequencing analysis was performed using HiSeq 2500 Rapid Run. Differentially expressed genes involved in glycolysis, PPP, and glutaminolysis were detected using EBseq. A false detection rate analysis with 0.05 threshold was performed and considered as biostatistic difference ($p < 0.05$). Data represent mean ± SD (n=3).

Table 2

Relative level of enzymes involved in oxidative phosphorylation in Cr(VI)-transformed cells and passage-matched normal cells

Gene Symbol	Mitochondrial complex	BEAS-2B	BEAS-2B-Cr(VI)
NDUFA13	Complex I	58.1 ± 6.2	34.9 ± 12.2
NDUFA11	Complex I	10.0 ± 0.3	6.4 ± 1.0
NDUFC2	Complex I	154.6 ± 10.8	81.3 ± 23.5
NDUFS8	Complex I	182.5 ± 2.5	99.2 ± 26.7
NDUFS7	Complex I	71.7 ± 3.8	46.4 ± 13.4
NDUFS5	Complex I	544.2 ± 77.2	271.8 ± 21.4
NDUFB7	Complex I	254.3 ± 20.1	153.6 ± 29.6
NDUFV1	Complex I	12.4 ± 4.0	5.8 ± 1.2
NDUFA6	Complex I	105.5 ± 3.4	0.5 ± 0.8
NDUFA2	Complex I	152.4 ± 26.5	72.6 ± 20.1
SDHA	Complex II	246.5 ± 45.9	105.6 ± 20.1
UQCRC1	Complex III	108.1 ± 8.3	84.5 ± 18.0
UQCR11	Complex III	160.3 ± 18.5	101.5 ± 4.1
UQCR10	Complex III	103.6 ± 11.4	61.4 ± 9.1
UQCRH	Complex III	42.0 ± 3.8	26.0 ± 2.1
COX4I1	Complex IV	11.6 ± 4.6	3.4 ± 1.3
COX6A1	Complex IV	1.4 ± 0.6	0.7 ± 0.1
COX6B1	Complex IV	6.4 ± 1.7	1.8 ± 0.7
ATP5H	Complex V	631.8 ± 39.9	362.8 ± 11.1
ATP5L	Complex V	119.5 ± 11.7	87.3 ± 19.9
ATP5C1	Complex V	76.2 ± 19.2	1.6 ± 0.6

Relative levels of enzymes involved in mitochondrial oxidative phosphorylation. The method used is the same as that in Table 1. Genes involved in mitochondrial oxidative phosphorylation were detected using EBseq. A false detection rate analysis with 0.05 threshold was performed and considered as biostatistic difference ($p < 0.05$). Data represent mean ± SD (n=3).