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Research Article

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Warburg Effect Reviewed: New Roles for Bifunctional Antidiabetic - Anticancer Active Hypoglycemics and Dysglycemics

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Abstract

Otto Heinrich Warburg first described the phenomenon that majority of cancer cells preferentially use aerobic glycolysis for energy production.

The key towards effective tumor control lies in understanding the Warburg effect and the interplay between the 3 key factors and genes involved in the regulatory processes (c-MYC, HIF-1 and p53 factors).

Hyperglycemia is the major link between cancer and diabetes. It is a key factor in chemoresistance due to its effects on DNA and dysregulation of tumor growth through inhibition of tumor suppression genes and increase in migration of tumor cells. It is also a major factor in initiation and sustenance of oxidative stress, an underlying common pathology of both cancer and diabetes.

Several antidiabetics have demonstrable anticancer effects in vitro and in vivo due to their roles in regulating glucose metabolism.

The aim of this review is to investigate the mechanisms through which the Warburg process sustains tumor growth and proliferation and how this process can be exploited and reprogrammed to disrupt tumor metabolism by severance of glucose and oxygen supply to tumor cells, while causing minimal dysregulation of normal tissue process.

This review equally aims to identify new roles for Antidiabetics and Dysglycemics in tumor control.

Introduction

The Warburg effect describes the phenomenon that majority of cancer cells preferentially use the less efficient aerobic glycolysis, used by normal cells under anaerobic conditions, than the normal tricarboxylic acid cycle and oxidative phosphorylation for energy production, with lactate as end product in the cytosol.

This discovery was first made by Otto Heinrich Warburg in 1924.To write it in his own statement: "Cancer, above all diseases, has countless secondary causes. But, even for cancer, there is only one prime cause. Summarized in a few words, the prime cause is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar."

In 1929, Herbert Crabtree discovered that there were variations in glycolysis, respiratory rates and fermentation in tumor cells and concluded that these changes could be due to genetic or environmental factors. Racker, Jeffrey Flier and Morris Birnbaum discovered that the process of aerobic glycolysis can be controlled by growth factor signaling [1].

Although, most cancer cells prefer glycolysis for ATP generation, they are also known to switch to other mitochondrial pathways including oxidative phosphorylation and Tricarboxylic acid cycle, through reprogramming once the need arises or if glycolysis is suppressed [2].

The Warburg metabolic process though made use of, is not peculiar to cancer cells alone but is equally seen in normal fast-growing cells. This effect can be visualized using Positron Emission Tomography (PET) Scanning, where patients are injected with 18F- fluorodeoxyglucose, which is avidly taken up by tumor cells and also by normal actively growing tissues. Majority of cancer cells are PET-positive, more for the actively growing ones [3].

Understanding the Warburg phenomenon appears to be the key towards effective and complete tumor control.

Several antidiabetics and dysglycemics have demonstrable anticancer effects in vitro and in vivo and this are not surprising.

The aim of this review is to investigate the mechanisms through which the Warburg process can be reprogrammed and applied to disrupt tumor metabolism by severance of glucose and oxygen supply to tumor cells, while causing minimal dysregulation of normal tissue process.

Genes And Transcriptional Factors Link in the Warburg Effect

1. P53: p53 is a very important tumor suppressor gene. It activates oxidative phosphorylation and inhibits glycolysis in cells. Mutations in p53 gene changes this balance and leads to oncogenic transformation [4].

Tumor-associated mutant p53 (mutp53) proteins have been found to stimulate the Warburg effect in cultured cells and mutp53 knock-in mice through promoting GLUT 1 translocation to plasma membrane. Inhibition of glycolysis in tumor cells disrupted the ability of mutp53 in causing tumorigenesis [5].

2. MYC: these are protein coding proto-oncogenes that consist of 3 related human genes MYC (c-myc), MYCL (l-myc) and MYCN (n-myc).

MYC oncogene is implicated in many human cancers. It encodes a transcription factor c-Myc, that connects changes in cellular metabolic processes to oncogenesis. Myc can regulate the expression of genes that codes for glycolytic enzymes like lactate dehydrogenase directly, or it can indirectly repress microRNAs miR-23a/b to promote glutaminase (GLS) protein expression and glutamine metabolism [6].

3. HIF: Hypoxia-inducible factors are a group of transcriptional factors (HIF - 1α and β, HIF-2 and HIF-3) that promotes oxygen delivery and adaptation to hypoxic microenvironment by regulating the expression genes linked to glucose uptake and metabolism (glucose transporters glycolytic enzymes, lactate production and pyruvate metabolism), angiogenesis, erythropoiesis, cell proliferation and apoptosis [7].

HIF-mediated Warburg effect has been linked to innate immune response to COVID-19 [8].

These are the 3 major factors implicated in the Warburg effect with their interplay.The control of energy metabolism is linked to these 3 transcription factors: c-MYC,HIF-1α and p53. Many oncogenes and tumor suppressor genes cluster link between glycolysis, Warburg effect and cancer cluster along the signal induction pathways that regulate these 3 transcription factors [9].

Dephosphorylated HIF-1 binds to p53 and prevents the downregulation of p53 by MDM-2 gene and hence allows it to promote apoptosis. Increased and sustained hypoxia activates p53-dependent apoptosis, that is initiated by stabilization of 53 by HIF-1 [10].

HIF and MYC have common target genes that both collaborate to induce; which include PDK1, LDHA, HK2 and TFRC, hence making them attractive therapeutic targets. A lot of tumors have gene changes, like MYC activation, that link with HIF to enhance their metabolic functions [11] [12].

4. Estrogen – related receptors (ERRs): EERs are orphan nuclear receptors that play important role in energy metabolism. They interact with Myc to enhance activation of transcription of genes encoding enzymes of glycolytic pathway. Their overexpression and repression lead to increase and decrease in aerobic glycolysis and lactate production [13]. They also function as cofactors of HIF and promote HIF – dependent transcription of genes of glycolytic enzymes under hypoxia [14].

5. Phosphoinositide 3-kinase (PI3k): PI3K/Akt (Akt is a serine/threonine kinase) a common proto-oncogenic pathway activation leads to enhanced glucose uptake and increase localization of GLUT1 in plasma membrane.

Studies showed that PI3K/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) as well as HIF-1 are central regulators of glycolysis, cancer metabolism and cancer proliferation [15].

6. SIRT 6 (Sirtuin 6): a stress responsive protein deacetylase expressed mainly in skeletal muscles, brain and heart and plays a role in aging, telomere care and glycolysis.

SIRT6 an epigenetic regulator of glycolytic genes is very much expressed in many cancers. Loss of SIRT6 or suppression by E2F1 (E2F transcriptionfactor1) promotes tumor growth by increasing glycolysis, making it an important target for cancer therapy [16] [17].

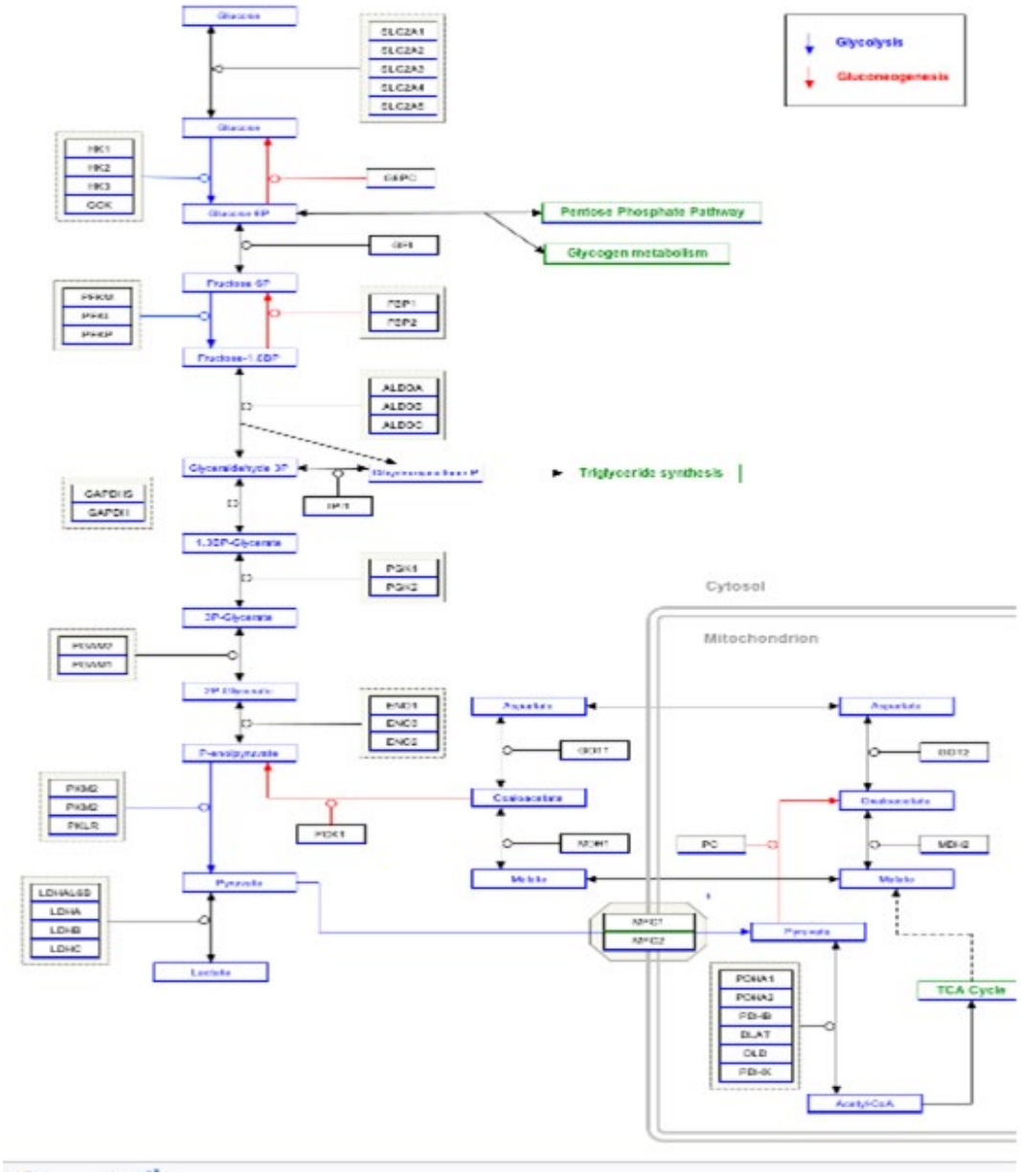
7. KRAS: The RAS family of oncogenes consist of HRAS, KRAS and NRAS. These are minute GTPases that switches between inactive GDP-bound and active GTP-bound states to control cell growth. KRAS promotes aerobic glycolysis by enhancing the expression and activity of the glucose transporters and glycolytic enzymes [18]. ADP-ribosylation factor 6 (ARF6) a minute GTPase which is a target of mutant KRAS induces cancer formation by promoting the Warburg phenomenon [19].

Vitamin C has been found to selectively kill colon cancer cells alone. Vitamin C activates RAS detachment from cell membrane inhibiting ERK 1 / 2 and PKM2 (pyruvate kinase M2) phosphorylation. This caused downregulation of GLUT-1 and PKM2 – PTB dependent protein kinase leading to inhibition of the Warburg phenomenon [20].

8. SRC: Proto-Oncogene, Non-Receptor Tyrosine Kinase. Src was found to be the major kinase that inhibit pyruvate dehydrogenase complex (PDH) through phosphorylation of tyrosine-289 of PDH E1α subunit (PDHA1) in cancer cells. Inhibitors of src reactivated PDH [21].

The main molecular process involved in Warburg phenomenon is poorly understood. Tumor suppressor p53 and oncogenes (SRC, AKT, RAS) are linked to the transcription factors HIF and oncogenic MYC. All the glycolytic enzymes have isoenzymes which are targets for HIF with hypoxia-response elements (HRE) in their promoter region [23].

FIGURE 1: Glucose Metabolism Interactive Pathways showing the key steps and enzymes, [22].



1. Glucose Transporters (GLUT 1-4): Glucose transporters 1 and 3 were the earliest to be discovered as HIF targets due to their enhancement of Glucose transport which facilitated the Warburg effect (23).

Studies have demonstrated very strong associations between GLUT 1 expression and the Warburg effect. The reversal of this expression in MNK45 culture cells reversed Warburg effect and induced apoptosis [24].

P38MAPK (Mitogen – activated protein kinases) activation in MNK45 cells greatly enhances GLUT-4 expression and increases glucose uptake and growth in gastric cancer cells. Inhibition of p38MAPK terminated the up-regulation of GLUT-4 [25].

STF -31 a selective inhibitor of GLUT 1 was found to selectively kill Renal Cell Carcinomas by targeting glucose transport via GLUT1, hence targeting the reliance of these cells on GLUT 1 for survival [26].

2. Hexokinase 2. (HK2): HK II catalyzes the number 1 irreversible step of glycolysis and it is usually overexpressed in tumor cells. Hexokinase II enhances Warburg effect by catalyzing the phosphorylation and transfer of the phosphate group of ATP to PDHA1 after converting Glucose to Glucose – 6- phosphate [27]. The normal human brain expresses mainly HK1, but Glioblastoma multiforme (GBM) has increased HK 2 expression resulting in rapid growth, drug resistance and intracranial invasion [28].

3. Phosphofructokinase (PFK-1): Analysis of the reduction that occur in each of the steps of glycolysis reveal that, out of the 3 rate - limiting glycolytic enzymes, phosphofructokinase isoenzymes provide the biggest chance as targets to block cancer cells from important energy and substrate sources, while enhancing

the growth and survival of normal cells [29]. TAp73, A structural homolog of the p53, is commonly overexpressed in human cancers. TAp73 activates the expression of PFKL which catalyzes the committed step in glycolysis and through this regulation TAp73 enhances the Warburg phenomenon [30].

4. Lactate Dehydrogenase A (LDHA): LDH is an oxidoreductase enzyme found in nearly all living tissues. It catalyzes the interconversion of pyruvate to lactate and NAD+ to NADH. It is a cytosolic enzyme released during tissue damage or injury, hence its prognostic and diagnostic roles in diseases. It has two subunits LDH A and LDH B and five isoforms LDH 1 – 5. LDH A is the key enzyme that drives and regulate the Warburg effect and hence the main target of new anticancer drug development.
Increase in activity of glycolytic enzymes and inhibition of TCA cycle enzymes lead to increase lactate associated with the Warburg effect. Increase lactate and acid-base change modification with acidification of tumor extracellular environment causes cancer to spread [31]. Human tumor suppressor folliculin (FLCN) binds to and uncompetitively inhibits LDHA. Cancer cells that demonstrated Warburg effect show FLCN dissociation from LDHA proving that shifts in glycolysis in cancer cells results from FLCN inactivation [32]. Some selenobenzene compounds like PSTMB inhibit LDHA and lactate production, without disrupting the expression of LDHA [33].
5. Pyruvate Kinase M2 (PKM2, M2-PK): This is the enzyme that catalyzes the last step of glycolysis.it is important in rapidly dividing tissues (embryonic development and wound healing) and overexpressed in many cancers where it plays a role in propagation and metastases. It has four isoforms PKL, PKR, PKM1 and PKM2.
PKM2 regulates HIF 1, interacts with HIF1 and activate the HIF-1α-dependent transcription of enzymes important for aerobic glycolysis in macrophages [34, 35].
6. Pyruvate Dehydrogenase Complex (PDC): Is a complex of 3 enzymes (E1, E2 and E3) in the mitochondria that converts pyruvate to acetyl-COA. It is inactivated through phosphorylation by Pyruvate dehydrogenase kinase complex which has 4 isoforms (PDK 1-4). PDH is a key mitochondria enzyme in energy metabolism and the main link and switch between glycolysis, TCA cycle and oxidative phosphorylation.
Drugs which inhibit PDH induces the Warburg effect and the overexpression of PDH subunits inhibits Warburg effect and induces apoptosis, making it a key enzyme in tumor control [36-40].

Therapeutic Advances and Recent Developments

1. Gene Therapy: The long-term effective control of cancer lies in advances in gene therapy directed against c-MYC, HIF-1 and p53 factors, commonly referred to as the 'triad of Warburg' using appropriate genetically engineered vectors.
Gene therapy programmes can be expanded and modelled after other National healthcare programmes.
2. Stem Cell research: The second most promising in the fight against cancer after gene therapy is stem cell research.
The induction of the Warburg effect leads to the enhancement of cancer stem cell (CSC) self-renewal and undifferentiation. HIF is overexpressed in hypoxic condition and activates the cascade of pathways in CSC metabolic reprogramming [41]. A novel hydrogel called a double network (DN) gel has been found to rapidly reprogramme differentiated cancer cells into cancer stem cells [42] [43].
3. Targeting Post-Translational Modifications [44].
4. Development of Biomolecules (Antibodies) Against Transcription Factors.
5. Targeting Cancer Metabolism: Some pharmacological compounds like 2-deoxy-D-glucose, Dichloroacetic acid and 3-bromopyruvate have been developed to inhibit the Warburg effect through disrupting cancer metabolism [45]. Other agents include SB-204990, 3-bromo-2-oxopropionate-1-propyl ester (3-BrOP), 5-thioglucose, Alpha-cyano-4-hydroxycinnamic acid.
a. Dichloroacetate (DCA) an analog of acetic acid reverses the Warburg effect by inhibiting the pyruvate dehydrogenase kinases (PDKs) and enhances oxidative metabolism of pyruvate [46].
b. 3 – Bromopyruvate an analog of lactic acid is an alkylating agent and potent inhibitor of glycolysis through selective alkylation of glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) [47, 48].

New Roles for Antidiabetics and Dysglycemics in Warburg Effect

Antidiabetics are known to have beneficial effects in the management of cancer patients, this is because hyperglycemia is the major link between diabetes and cancer.

Studies have shown that hyperglycemia directly and indirectly damages the DNA, and causes the process of tumor control to go unregulated, inhibit tumour suppressor genes and causes oncometabolites to accumulate. Hyperglycemia also increase migration and inhibits apoptosis in cancer cells making them more aggressive and resistant to chemotherapy [49].

Many conventional cytotoxics and hormonal chemotherapeutics cause hyperglycemia as adverse effects and this has serious consequences on their effectiveness in prolonged use. Cytotoxics cause hyperglycemia and insulin resistance through direct actions on glucose transporters, while hormonal chemotherapeutics through indirect hormone mediated pathways of raised ACTH, Cortisol etc.

1. Conventional Antidiabetics with known Anticancer Effects:
Metformin has been found to sensitize cancer cells to Radiotherapy and Chemotherapy and potentiate the effects of Chemotherapeutic agents [50-52].

Metformin and Troglitazone (thiazolidinedione) have been found to prevent cancer risks in diabetic patients [53]. Troglitazone showed in vitro and in vivo cytotoxicity in two pancreatic cell lines [54]. Anticancer effects have also been showed for glimepiride on breast cancer cells [55].

Insulin potentiates the anticancer activity of 5-FU when it is treated before 5-FU for the appropriate time in human esophageal and colonic cancer cell lines [56].

Metformin has been shown to have anti-cancer effects in various hormone-sensitive tumors, such as breast cancer, pancreatic cancer, colon cancer, and prostate cancer (PCa) [57].

Metformin has been shown to be involved in the regulation of insulin/insulin-like factor. It also. Activate AMPK through the tumor suppressor LKB1. Activation of AMPK leads to increase in oxidative metabolism and inhibition of anabolism [58, 59].

Despite the potent anticancer properties of conventional antidiabetics, their role in Warburg effect have not been given serious attention. Apart from few studies with metformin, little or nothing is known about effect of other antidiabetics on Warburg effect and the therapeutic potentials in this regard.

2. Dysglycemics with known known Anticancer Activities: these are drugs that cause both hyperglycemia and hypoglycemia as part of their pharmacological effects. Examples are Beta blockers Propranolol, Atenolol, Metoprolol and Fluoroquinolones Ciprofloxacin, Gatifloxacin (most likely to cause dysglycemia) and Moxifloxacin (least likely to cause dysglycemia).
Propranolol was found to sensitize prostate cancer cells to glucose, inhibit prostate cancer cell growth, induces apoptosis and changes mitochondria metabolism [60, 61].
Gatifloxacin inhibit the growth of MIA PaCa-2 and Panc-1 cells by causing S and G(2)-phase cell cycle arrest without induction of apoptosis. Gatifloxacin mediated G(2)-phase cell cycle arrest was through p53 in the 2 cell lines [62].
3. Hypoglycemics with known Anticancer Activities: These include Quinine and Aspirin.
4. Hyperglycemics with known Anticancer Activities: These include Steroids like Dexamethasone known for its potent apoptotic effects and the Statins Simvastatin, Atorvastatin, Pravastatin etc.

Research Proposal

Research Title: To Investigate In Vitro the Effects of Antidiabetics and Dysglycemics with Anticancer Properties on the Key Enzymes of Glucose Metabolism Linked to the Warburg Effect.
Research Aim: To Identify the Roles of Antidiabetics and Dysglycemics with Anticancer Properties in the Warburg Phenomenon, through their Activities on the Key Enzymes of Glucose Metabolism (Lactate dehydrogenase).

Materials And Method

Materials

1. Standard Human Breast Cancer Cell Lines: MCF-7, MDA-MB-468, T-47D.
2. Cancer cell line culture media materials
a. Sera – Fetal Bovine Serum (FBS) containing cell growth media (Glucose/Glucosamine, Amino acids, Vitamins, ionic solution etc)
b. Reagents – Phosphate buffer solution, Trypan blue, Trypsin, 70% alcohol.
c. Consumables eg Cell culture flasks, Centrifuge tubes, pipettes, hand gloves, etc
3. Cell culture laboratory equipment
4. Various dilutions of pharmacological agents using known solvents.
5. Lactate dehydrogenase assay kits
6. Lactate concentration quantification assay kits

TABLE 1: Showing Drugs with known anticancer activities that alters glucose metabolism and conventional cytotoxics with little or no effects on glucose metabolism.

Drug	Example	Example			
Antidiabetics	**Rosiglitazone**	**Glibenclamide**			
Dysglycemics	**Propranolol**	**Gatifloxacin**			
Hypoglycemics	**Quinine**	**Aspirin**			
Hyperglycemics	**Dexamethasone**	**Simvastatin**			
Cytotoxics	**Cyclophosphamide**	**Paclitaxel**			

Note that Metformin, an antidiabetic with very promising and well researched anticancer activities is not used in this proposed study because of its propensity to cause severe lactic acidosis. Although, Metformin induced lactic acidosis is not a direct effect of the drug on cell metabolic processes, but rather due to its hepatic inhibition uptake of lactate. It will be safe to leave it out of this proposed study.

Method

A) The IC50 (the concentration the inhibit cell viability by 50%) of the various pharmacological agents are first determined using the Trypan blue exclusion method (mitochondrial metabolism methods include MTT, MTS, XTT, WST-1 assays)
B) The effect of the drugs on LDH can be measured by quantifying the LDH activity in the culture media, containing the IC50 of the various drugs gotten from Trypan Blue exclusion method, using the LDH assay kit.
This assay makes use of the fact that LDH reduces NAD to NADH, which is specifically detected by colorimetric spectrophotometer at
specific range of absorbance.
Cytotoxicity is quantified in 2-step, colorimetric reaction.
Step 1: lactate dehydrogenase (LDH) catalyzes the reduction of NAD+ to NADH and H+ by oxidation of lactate to pyruvate.
Step 2: Diaphorase uses the NADH and H+ to catalyze the reduction of a tetrazolium salt (INT) to colored formazan, that absorbs strongly at 490 – 520nm. The amount of formazan formed is proportional to the amount of LDH released into the culture medium due to cytotoxicity [63].

Expected Findings and Conclusion

The cytotoxicity profiles of the drugs are gotten both from their IC50 values and from the quantification of LDH.

The effect of each and different classes of the pharmacological agents on the lactate dehydrogenase enzyme in the media can be estimated by comparing the various absorbance values gotten from the colorimetric study described as time 0 with colorimetric values at time 4, 8, 12, 24, 48 hours and also comparing it with the absorbance in media not containing any pharmacological agent.

Lactate concentration in the culture media at different time intervals (0, 4, 8, 12, 24, 48 hours) can equally be used for the evaluation of the activities of these drugs in Warburg effect.

Conclusion

Understanding the Warburg phenomenon appears to be the key towards effective and complete tumor control. The three main factors and genes involved in the Warburg phenomenon are c-MYC, HIF-1 and p53 factors.

The interplay between these factors and the key enzymes of glucose metabolism, other regulatory factors and signaling pathways provides a means towards understanding the Warburg concept and role it plays in linking diabetes and cancer.

Antidiabetics are known to have beneficial effects in the management of cancer patients, this is because hyperglycemia is the major link between diabetes and cancer.

New roles have been assigned to antidiabetics and Dysglycemics with known anticancer properties, due to their beneficial properties in the regulation of the Warburg phenomenon.

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