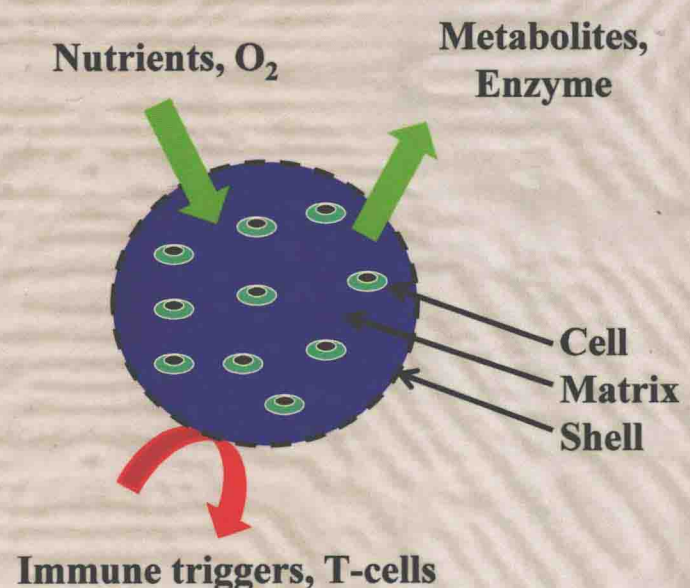
Materials for Biomedical Applications



Edited by
Mohammad A. Jafar Mazumder and Amir Al-Ahmed

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Materials for Biomedical Applications

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Preface

Biomedical applications of materials as a form of micro to macro molecules provides an outstanding demonstration of the multi- and interdisciplinary arena of materials. The aim of this book is to provide a critical insight on scientific, engineering and processing aspects of various materials, which can ultimately contribute towards the advancement of medical sciences.

As the target audiences cover a wide interdisciplinary field, each peer-reviewed chapters written with detail background by a selected group of academic and clinical experts. This book entitled "**Materials for Biomedical Applications**" reflect the true inter-disciplinary nature of materials science, demonstrate the scientific background and interaction between the materials and biosystems, biocompatible or biodegradable polymers, materials for diagnostic, and the development of devices and enabling technologies for therapeutic applications.

This book summarises the up-to-date status of the field, covers important scientific and technological developments by many distinguished experts, who came together to contribute their research work and comprehensive, in-depth and up to date articles. Written in a versatile and contemporary style, this book can be used as an invaluable reference source for graduate students, scientist, researcher working in chemistry, polymer chemistry, polymer engineering, chemical engineering and materials science. We are thankfully appreciate the tremendous efforts and co-operation of all contributing authors for their devotion, valuable time in preparing state-of-art chapters for this book. We would also like to express our gratitude to the publishers and all authors, and others for granting us the copyright permissions to use their illustrations. Although sincere efforts were made to obtain the copyright permissions from the respective owners to include the citation with the reproduced materials, we would like to offer our sincere apologies to any copyright holder if unknowingly their right is being infringed.

For acknowledgment, among the editors, Dr. Mohammad A. Jafar Mazumder would like to take this opportunity to express his sincere thanks to Dr. Abdullah J. Al-Hamdan (Chairman, Department of Chemistry, KFUPM) and also to his colleagues at the King Fahd University of Petroleum & Minerals, Saudi Arabia for their endless support and co-operation.

Dr. Amir Al-Ahmed, would like to take this opportunity to express his sincere thanks to Dr. Haitham M. Ba-Haidarah (Director CORE-RE, KFUPM) and also to his colleagues at the King Fahd University of Petroleum & Minerals, Saudi Arabia for their never-ending support and co-operation.

Without their continuous encouragement, this book would have not been brought into its final form. We would also like to acknowledge the sincere efforts of Mr. Thomas Wohlbier of TTP publishing Authority, in evolving this book into its final shape.

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Pentose Phosphate Pathway in Disease and Therapy

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Keywords: Pentose phosphate pathway, glucose-6-phosphate dehydrogenase, transketolase, NADPH, nucleotide biosynthesis, glutathione, metabolic diseases, non-metabolic diseases, cancer, epilepsy, Alzheimer's disease, chemotherapy, gene therapy, chemoresistance, metabolomics, metabolic flux analysis, novel drug discovery and development.

Abstract. Pentose phosphate (PP) pathway, which is ubiquitously present in all living organisms, is one of the major metabolic pathways associated with glucose metabolism. The most important functions of this pathway includes the generation of reducing equivalents in the form of NADPH for reductive biosynthesis, and production of ribose sugars for the biosynthesis of nucleotides, amino acids, and other macromolecules required by all living cells. Under normal conditions of growth, PP pathway is important for cell cycle progression, myelin formation, and the maintenance of the structure and function of brain, liver, cortex and other organs. Under diseased conditions, such as in cases of many metabolic, neurological or malignant diseases, pathological mechanisms augment due to defects in the PP pathway genes. Adoption of alternative metabolic pathways by cells that are metabolically abnormal, or malignant cells that are resistant to chemotherapeutic drugs often plays important roles in disease progression and severity. Accordingly, the PP pathway has been suggested to play critical roles in protecting cancer or abnormal cells by providing reduced environment, to protect cells from oxidative damage and generating structural components for nucleic acids biosynthesis. Novel drugs that targets one or more components of the PP pathway could potentially serve to overcome challenges associated with currently available therapeutic options for many metabolic and non-metabolic diseases. However, careful designing of drugs is critical that takes into the accounts of cell's broader genomic, proteomic and metabolic contexts under consideration, in order to avoid undesirable side-effects. In this review, we discuss the role of PP pathway under normal and abnormal physiological conditions and the potential of the PP pathway as a target for new drug development to treat metabolic and non-metabolic diseases.

Introduction

Every living organism, whether it is an unicellular prokaryote or a multicellular eukaryote, possess metabolic pathways to break-down large organic molecules into small intermediary compounds, to generate energy for cellular synthesis [1]. While thousands of enzymes, substrates and co-factors are involved in these reactions, anomalies of the metabolic pathway often affect the life-style of the most complex and multicellular organism on earth, i.e., human. Human being combats much different type of diseases throughout their lifetime. Human disease is defined by impairment of normal functioning because of disordered or abnormal conditions of an organ or the whole body, which may results from the effects of genetic or developmental errors, infection, nutritional deficiency, toxicity, or other unfavourable environmental factors [2]. The cause and effect of disease are multifaceted. Many diseases are caused by pathogenic microorganisms (e.g., virus-influenza, fever, bacteria-tuberculosis,

diarrhoea; parasites-malaria, dengue), while others are due to mutation in the genetic element (e.g., autoimmune disease-Systemic lupus erythematosus) or both (e.g., cancer). The effect of the disease can be either acute or chronic. However, the severity of the disease can be life threatening, if it remains undetected or untreated.

During the early 20th century, invention of the antimicrobial drug Penicillin, saved lives of thousands of soldiers and civilians from syphilis, staphylococcal and streptococcal infections [3]. New drugs, vaccines, diagnostics and surveillance systems against epidemic diseases like malaria, cholera, pneumococcal infection, tuberculosis, sexually transmitted diseases (STDs) and severe acute respiratory syndrome (SARS) saved thousands of lives worldwide. Insulin and chemotherapy drugs save or increase the longevity of thousands of lives that are suffering from non-infectious diseases such as diabetes or cancer. Malaria and cholera caused by parasites and bacteria, respectively, used to appear in epidemic forms in many developing countries of South-East Asia [4–8]. On the other hand, diabetes, cancer, Alzheimer's disease, etc., that are associated with genetic factors or other stimuli are now global problem as these have turned into an epidemic form in many developed countries [9-11]. Whatever the cause of the disease is, treatment of diseases certainly impose socio-economic burden for the affected countries. Unfortunately, in recent years, an additional cost has been added to the existing costs associated with the management of human diseases is drug resistance. Not only pathogenic bacteria, but even cancer cells are showing drug resistance properties, causing relapses at later stages of treatment. As a result, it has become highly important to develop new drugs or modify existing treatment strategies.

Design and development of novel drugs require knowledge on the metabolic pathways of the infected cells as well as disease causing organisms, whenever applicable [12]. Researchers already identified that many pathogens or even cancer cells adopt alternative pathways for nucleotide synthesis and cell metabolism under diseased conditions. Traditional therapies or new targeted therapies mend abnormal functions of single genes and proteins, as well as affect a narrow range of metabolic downstream reactions. However, metabolic networks inherently possess wide functional flexibility due to the presence of multiple alternate macromolecule synthetic pathways. As a result, targeted drugs may fail to control a pathologic phenotype that eventually develops drug resistance. To overcome these problems, detailed information on the metabolic pathways could pave a solution to the problem of 'drug resistance' for the drug developing companies.

Use of next generation sequencing technology and proteomics has advanced our understanding of the metabolomics under pathological conditions. Although these data are highly informative, additional information on the cell's energy state or co-factors might provide useful information under pathological conditions. In this context, metabolic flux analysis (MFA) is a robust technique to understand the biological reactions. MFA applies tracers (e.g., ^{13}C labelled glucose or acetate or ^{14}N labelled glutamine) to detect and calculate the metabolic state of cells [12-14]. In the field of metabolic engineering, this has been applied vastly for strain improvement of biotechnologically important prokaryotic or simple eukaryotic organisms to understand the effect of genetic alterations, changes of external conditions and different nutritional status. Since, mammalian cells exert heterogeneous nature under diseased condition, several tracers are applied to obtain information on diverse metabolites and these data are integrated to the transcriptomic and proteomic data to unravel the correct pathway under pathological condition. Perhaps a similar approach can be applied to narrow down metabolic pathways associated with drug resistance, as well.

This review is focussed to discuss the attributes of one of the most important metabolic pathways known as the pentose phosphate (PP) pathway, and human diseases and therapy associated with this pathway. This is the only pathway for nucleotide biosynthesis in both prokaryotes and eukaryotes. There are now ample evidences from in vitro studies that suggest that cancer cells or drug resistant organisms utilize PP pathway for abnormal proliferation or biomass accumulation [15-17]. Defects in the enzymes or genes of this pathway have already been known to be associated with inborn error, heritable or non-heritable diseases, and even with colon cancer, breast cancer etc. Therefore, PP pathway could be a target for new drug development or for modification of existing drug therapies. Therapeutic approaches to correct the mutation of a particular gene or several genes using gene

therapy or introduction of small molecule inhibitors or combinations could be promising agents to treat the drug resistance properties of cancer cells.

Overview of the Pentose Phosphate Pathway

Pentose phosphate pathway is present in every living organism. The history of the metabolic role of this pathway dates back to 1926. Patients treated with the malarial drug primaquine led to the first medical description of a drug- induced hemolytic anemia that correlated with an intrinsic defect of red blood cell metabolism. In 1948, glucose-6P-dehydrogenase, the first rate limiting enzyme of the PP pathway was discovered. In 1960, this pathway was conceived as part of glucose metabolism, based on studies on enzymatic activities and metabolites in yeast [18]. Now it is well established that once glucose enters the cell, it can be used in three major pathways: glycogen synthesis, glycolysis and the PP pathway. While glycogen is used for glucose storage, glycolysis is considered as the key metabolic pathway for glucose metabolism and requires oxygen to generate energy in the form of ATP, NADH and pyruvate. On the other hand, only a very small fraction of glucose (5-30%) is metabolised through the PP pathway. The interplay between the glycolytic pathway and the pentose phosphate pathway are highly dependent on the metabolic state and growth rate of the organism. In human being, PP pathway is most active in liver, mammary glands and adrenal cortex of the brain [19].

Branches of the PP Pathway

Cytoplasm or cytosol is the site where pentose phosphates are synthesized from hexose sugars through a series of biochemical reactions. Biochemically, the pentose phosphate pathway is divided into two branches: oxidative branch and the non-oxidative branch. The oxidative branch is operated by three enzymes: glucose 6-phosphate dehydrogenase (G6PDH), 6-phospho gluconolactonase (6PGL) and 6- phosphogluconate dehydrogenase (6PGDH). The substrate of the oxidative branch, glucose-6-phosphate (G6P), is generated from the glycolytic pathway and is oxidized into 6-phosphogluconolactone by G6PDH with the production of NADPH. The unstable lactone ring is then opened by lactonase into 6-phosphogluconic acid and undergoes oxidative decarboxylation by the 6PGDH enzyme, which irreversibly produces ribulose-5-phosphate and a second NADPH and CO_2. The resulting ribulose-5-phosphate can be further converted into ribose-5-phosphate and used for the synthesis of nucleotides or can be converted into xylulose-5-phosphate and fed into the non-oxidative branch of the pentose phosphphate pathway [20] (Fig. 1).

The non-oxidative branch of the pentose phosphate pathway is operated by 4 different enzymes synthesizing 3-7 carbon containing molecules. Unlike the oxidative branch, enzymes of the non-oxidative branch take part in reversible reactions and this occurs mainly during the inter-conversion of ribulose-5-phosphate into ribose-5-phosphate or ribulose-5-phosphate into xylulose-5-phsophate. Enzymes of the non-oxidative branch are ribose-5-phosphate isomerase (R5PI), ribose-5-phosphate epimerase (R5PE), transketolase (TK) and transaldolase (TA). R5PI and R5PE convert ribulose-5-phosphate, synthesized at the oxidative branch, into ribose-5-phosphate and xylulose-5-phosphate, respectively. Ribose-5-phosphate and xylulose-5-phosphate are then catalyzed by TKs into glyceraldehyde-3-phosphate (3- carbon) and sedoheptulose-7-phosphate (7-carbon). These two intermediates are further catalyzed by TAs into fructose-6-phosphate and erythrose-4-phosphate. Erythrose-4-phosphate then reacts with a second xylulose-5-phosphate mediated by the TKs, into the final products fructose-6-phosphate and glyceraldehyde-3-phosphate. These final products are either recycled into the glycolytic pathway for energy production or reintroduced into the PP pathway for biosynthetic reactions [20] (Fig. 1).

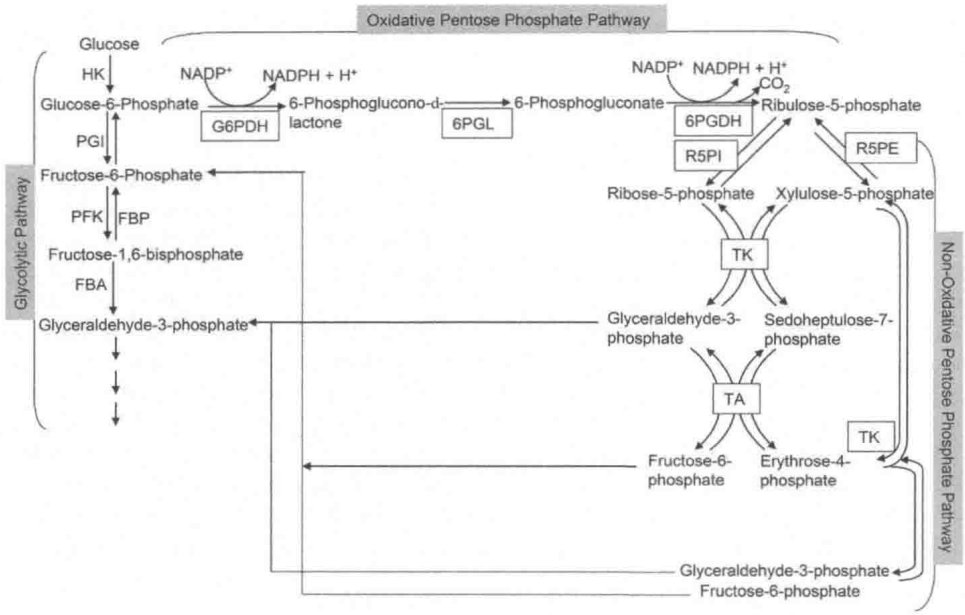

Fig. 1. Different branches of pentose phosphate pathway and their links to the glycolytic pathway. G6PDH: Glucose-phosphate dehydrogenase; 6PGL: 6-phospho glucono lactonase; 6PGDH: 6-phosphogluconate dehydrogenase; R5PI: Ribose-5-phosphate isomerase; R5PE: Ribose-5-phosphate epimerase; TK: Transketolase; TA: Transaldolase; HK: Hexokinase; PGI: Phosphoglucose isomerase; PFK: Phosphofructo kinase; FBP: Fructose bisphophatase; FBA: Fructose bisphosphate aldolase.

Functions of the PP Pathway

Metabolites synthesized in the PP pathway act as precursor molecules for macromolecule biosynthesis such as vitamin, thiamine or co-factor biosynthesis, nucleotide biosynthesis, aromatic amino acid and other amino acid biosynthesis, fatty acid metabolism or cholesterol biosynthesis, steroid hormone synthesis (Table 1). The PP pathway is also utilized for drug metabolism or detoxification reactions. In addition, NADPH synthesized through the oxidative branch, maintains a highly reducing environment to protect cells from oxidative damage. In fact, this is the main pathway for the maintenance of reducing environment during biosynthetic reactions, and for the supply of ribose sugars for the biosynthesis of nucleotides or the constituents of cellular genetic materials [16, 20-22].

Table 1. Importance of the Pentose Phosphate Pathway in cellular biosynthesis [16, 20-22].

Branches of the PP pathway	Products or intermediate compounds of PP PATHWAY	Biological Function
Oxidative branch	NADPH	Provides reducing power for biosynthetic reactions ii) Protects cells against oxidative stress, either by neutralizing reactive oxygen species (ROI) or indirectly via regenerating reduced glutathione (GSH) from its oxidized form GSSG which is previously produced by GSH peroxidase (GSHPx)-catalyzed reactions Utilized by the cytochrome P_{450} monooxygenase system to degrade xenobiotic compounds or drugs
Non-oxidative branch	Pentose-5-Phosphates: Ribose-5-P 2'deoxy ribose-5-P 5-phosphoribosyl-1-pyrophosphate (PRPP)	i) Structural components of nucleotides: a. Basal structural component of RNA b. Basal structural component of DNA c. Precursor of both de novo and 'salvage' synthesis of nucleotides ii) Intermediate products of purine metabolism and act as precursor molecules of cofactors, e.g., riboflavin, flavin mononucleotide (FMN), flavin adenine di nucleotide (FAD) iii) Precursor of the amino acid, Histidine.
	Erythrose-4-Phosphate	i) Precursor of vitamin B6. ii) Precursor of aromatic amino acid.
	Sedoheptulose-7-P	Important constituent of bacterial cell wall.
	Fructose -6-P	Recycled to the glycolytic pathway to maintain the intracellular level of Glucose-6P, which primes both the glycolytic and PP pathway and required for storage of glycogen or starch.
	Glyceraldehyde-3-P	Recycled to the glycolytic pathway for ATP synthesis.

Regulation of the PP Pathway

Metabolic flux analysis of the substrates of the two branches of PP pathway showed that the flux of the oxidative part of the PP pathway is about 14 times higher than that of the nonoxidative part in brain. Again, high ratios of oxidative versus nonoxidative part of the PP were found for the mammary gland (33:1), adipose tissue (20:1), lung (11:1), and skeletal muscle (9:1) [20-21, 23-24]. These clearly indicate that cells require significantly high reducing environment for biosynthetic reactions and for the optimum activities of cells, the two branches must be stringently regulated. Indeed, two enzymes, glucose-6-phosphate dehydrogenase (G6PDH) and transladolase (TA) act as the rate limiting enzymes for this pathway.

G-6-phosphate-dehydrogenase. *G-6-phosphate-dehydrogenase* is the first rate limiting enzyme of the PP pathway. The gene is located in the long arm of the X-chromosome at position 28. Its function was first reported in 1948 in catalyzing the first reaction in the PP pathway, providing reducing equivalents (NADPH) to all cells and defence against oxidative stress [20]. The enzyme functions as a dimer or tetramer in human cells where each monomer is composed of 515 amino

acids. G6PDH is considered as constitutively expressed housekeeping enzyme and it controls the entry of the metabolite, glucose-6-phosphate (G6P) from glycolytic pathway into the PP pathway. The activity of this enzyme is dependent on the availability of G6P and intracellular concentration of $NADP^+$ and NADPH. In addition, several genes also regulate the activity of this enzyme.

Regulation of G6PDH by NADPH Level. Unstressed cells have a higher ratio of $NADPH/NADP^+$. $NADP^+$ is required to stabilize G6PDH to its proper conformation and increases the activity of the enzyme under conditions that leads to increased NADPH formation. NADPH, on the other hand, destabilizes the enzymes conformation, lowers its stability, impairs folding and affects the kinetic parameters of the enzyme, such as concentration of G6P, $NADP^+$, pH and affinity for substrate. A variety of reactions affect the level of NADPH in cell which eventually lowers the ratio of $NADPH/NADP^+$ and modulates the activity of G6PDH. NADPH serves as the reducing equivalent for a number of reactions in the metabolic pathway. These include: glutathione (GSSG) reduction and peroxide disposal, fatty acid biosynthesis, cholesterol biosynthesis, nitric oxide (NO) production by NO synthase, superoxide production by NADPH oxidase, hydroxylation reactions, degradation of heme, polyol metabolism and the thioredoxin system [20, 23].

Glutathione (GSSG) Reduction and Peroxide Disposal. Reactive oxygen species (ROS) such as peroxides and oxygen radicals are continuously generated during aerobic metabolism in red blood cells or during oxidative fatty acid metabolism. These ROS are responsible for cellular damage such as lipid peroxidation, DNA strand breakage and protein inactivation. Glutathione (GSH) is an important component of the anti-oxidative system, being present at the mM concentration level in cell. GSH reacts directly with the ROS radicals and it is the electron donor in the reactions catalyzed by GSH peroxidises (GPx). The product of the oxidation of GSH by GPx is glutathione disulfide (GSSG). Within cells, GSH is regenerated from GSSG in the reaction catalyzed by glutathione reductase (GR), a flavoenzyme that transfers electrons from NADPH to GSSG. The cycling of GSH in the reactions catalyzed by GPx and GR depends on the availability of reduction equivalents in the form of NADPH. NADPH consumed during this process is regenerated by reactions catalyzed by G6PDH and 6-phosphogluconate dehydrogenase. Thus, clearance of peroxides from cellular system requires effective generation of NADPH and this is controlled by the GSH level [20, 23].

Fatty Acid Synthesis. In human fatty acids synthesis is highly active in liver, mammary glands and brain. Fatty acid biosynthesis occurs in the cytosol through a series of reactions where acetyl-CoA (synthesized from pyruvate, the end product of glucose metabolism) and Malonyl-CoA are linked to form palmitoyl-CoA. During this elongation process, NADPH supplies electrons to Malonyl-CoA [20, 23].

Cholesterol Biosynthesis. Cholesterol is an essential structural component of animal cell membrane, required to establish proper membrane permeability and fluidity. It is also a precursor of steroid hormones (e.g., progesterone, testosterone, estradiol and cortisol), bile acids and vitamin D. Cholesterol is synthesized from acetyl-CoA in three stages and NADPH acts as the electron donor for the first stage during which 3-hydroxy-3-methylglutaryl CoA (HMG) is converted to mevalonate [20, 23].

Nitric Oxide (NO) Production by NO Synthase. NO radicals are important signalling molecule and involved in numerous biological functions, including glutathione (GSH) synthesis (GSH), mentioned above. NO is the product of the reaction that is catalyzed by NO synthases (NOSs). Different isoforms of NOS are available in mammalian system where they are encoded by different genes. The constitutive NOS - 1 and NOS - 3 produce NO only for short periods, after activation by a raise in intracellular concentration of calcium ion (Ca^{2+}). In contrast, the inducible NOS - 2 causes a long lasting generation of high amounts of NO in the form of peroxynitrite. All isoforms of NOS need NADPH as an electron donor to produce NO and citrulline from arginine and molecular oxygen. Since NO production and expression of constitutive or inducible NOSs have been reported for neurons and different types of glial cells NADPH consumption by NOSs contributes to the regulation of the G6PDH enzyme by NO level [20, 23, 25].

Superoxide Production by NADPH Oxidase. NADPH oxidase (Nox) is a multi-subunit protein complex that uses electrons derived from NADPH to reduce molecular oxygen to superoxide. Activation of cells leads to the association of cytosolic protein subunits of Nox with the membrane-associated proteins to form the active complex that produces superoxide [20, 23].

Hydroxylation Reactions. A large number of drug-metabolizing enzymes that consist of cytochromes P450 and NADPH-dependent cytochrome P450 reductase have been reported for brain cells. The NADPH-dependent enzymes are involved in the hydroxylation of neurosteroids, the regulation of brain cholesterol homeostasis, the elimination of retinoids, and the metabolism of xenobiotics [20, 23].

Degradation of Heme. Two enzymes in heme degradation require reduction equivalents from NADPH. These are: heme oxygenase (HO) and biliverdin reductase. HO metabolizes heme to biliverdin, iron, and carbon-monoxide (CO). The latter one acts as a neurotransmitter. Biliverdin reductase, on the other hand, reduces biliverdin to bilirubin. While, bilirubin is toxic for the brain of newborns, NADPH-dependent recycling of bilirubin by biliverdin reductase plays some antioxidative role in the cells [20, 23].

Polyol Metabolism. During polyol metabolism, the enzyme aldose reductase uses NADPH as substrate to reduce glucose to sorbitol. Sorbitol is further oxidized to fructose by sorbitol dehydrogenase. Flux through the sorbitol pathway is present in the brain and bypasses the enzyme hexokinase, which is the control point of glucose metabolism. An elevated flux through the sorbitol pathway has been reported in the brains of diabetic patients. It is thought that depletion of cellular NADPH due to exaggerated flux through aldose reductase contributes to oxidative stress-mediated dysfunctions of neural cells in diabetes [20, 23].

Thioredoxin System. Thioredoxin and thioredoxin reductase are important constituent of the intracellular redox environment. Thioredoxin reductase is homologous to glutathione reductase (GR) and depends on NADPH as electron donor. The thioredoxin system can repair peroxynitrite induced disulfides in brain tubulin and therefore may be of high importance for brain cells under pathological conditions [20, 23].

G6PDH Regulation by Regulatory Genes. p53 is a transcription factor and a well known tumor suppressor gene. In cell, it plays important role in glucose metabolism and oxygen transfer. p53 directly binds with G6PDH and inhibits its function. Although the exact mechanism of p53 controlling G6PDH is not unravelled, frequent mutation of p53 and a high PP flux has been reported. Such mutations also make cells resistant to ROS. In addition to p53, TAp73, a structural homologue of the tumor suppressor gene p53 also regulates metabolism in tumor microenvironment and contributes to oncogenic cell growth. However, unlike p53, TAp73 is rarely mutated and frequently overexpressed in human tumours. TAp73 activates the expression of G6PDH and increases PP flux to direct glucose-6-phosphate to the production of NADPH and ribose-5 phosphate for the synthesis of macromolecules and detoxify reactive oxygen species (ROS) in tumor environment [26].

Non-steroidal Anti-inflammatory Drugs (NSAIDS). NSAIDs, such as ketoprofen decreases G6PDH activity. The underlying mechanisms is that, acyl-CoA, a derivative of many NSAIDs, interact with the same site that binds phosphoenol-pyruvate (PEP), a negative allosteric modulator of G6PDH. In addition, arachidonic acid, which is the precursor of prostaglandin and thromboxane (inflammatory response components) also regulate G6PDH by impairing the splicing efficiency of G6PDH pre-mRNA, via the activation of adenosine monophosphate activated protein kinase [20, 23].

Transaldolase (TA). *Transaldolase (TA)* is the rate limiting enzyme of the non-oxidative branch of the PP pathway. Human TA is located in chromosome 11 and the enzyme is encoded by a single-copy gene with 336 amino acids and a molecular weight of 38 kDa. The activity of this enzyme is dependent on the availability of its substrate ribulose-5-phosphte and inhibited by inorganic phosphate (Pi), D-arabinose-5-phosphate and glyceraldehyde-3- phosphate [20, 23]. Both, G6PDH and TA are also regulated by the glycolytic metabolites 2, 3-bisphosphate and glyceraldehyde-3-phosphates. These metabolites interconnect the PP pathway with glycolysis and gluconeogenesis and therefore, their concentration modulates the activities of G6PDH and TA [20, 23].

Diseases of the PP Pathway

Diseases associated with metabolic pathways are commonly called metabolic diseases. However, PP pathway has been associated with numerous types of diseases including cancer or malignancy. Thus PP pathway diseases can be classified into two major groups: non-malignant diseases and malignant diseases.

Non-malignant Disease. Non malignant diseases with respect to PP pathway can again be divided into two groups: metabolic diseases and non metabolic diseases.

Metabolic Diseases. Metabolic diseases are directly associated with defects on the enzymes of the particular metabolic pathways. In case of the PP pathway, defect or mutation of the oxidative branch and the non-oxidative branch enzymes have been reported, leading to abnormal accumulation or depletion of metabolites or reduced enzyme activity associated with the pathway, which is reflected in disease phenotypes.

Glucose-6-phosphate Dehydrogenase (G6PDH) Deficiency. G6PDH is present in almost all living organisms and it is expressed in all tissues and cell types from higher animals and plants to prokaryotic system. Diseases associated with reduced functioning of this enzyme are the most common heritable human enzyme defect, present in more than 400 million people worldwide [27-28]. Although most patients with the G6PDH deficiency are asymptomatic, symptomatic patients are almost exclusively male, due to the X-linked pattern of inheritance, but female carriers can be clinically affected due to unfavorable lyonization, where random inactivation of an X-chromosome in certain cells creates a population of G6PDH-deficient red blood cells coexisting with normal red cells. More than 100 missense mutations in the *G6PD* gene are known to date. The two variants (G6PDH A− and G6PDH Mediterranean) are the most commonly inherited variants. G6PD A− has an occurrence of 10% among American blacks, while G6PDH Mediterranean is prevalent in the Middle East. The known distribution of the disease is largely limited to people of Mediterranean origins (Spaniards, Italians, Greeks, Armenians, and Jews). Most of these mutations cause little or no disease. However, some mutations cause severe instability of the dimeric molecule, G6PDH. As a result, the patients suffer from lifelong chronic nonspherocytic hemolytic anemia (CNSHA). CNSHA patients are sensitive to drugs or chemicals that induces oxidative stress such as: (i) fava beans (leading to favism), (ii) antimalarial drugs, e.g. primaquine, pamaquine, and chloroquine; (iii) antimicrobial agents, e.g. sulfanilamide, sulfamethoxazole, and mafenide, (iv) thiazolesulfone, (v) methylene blue, (vi) naphthalene, (vii) certain analgesics, e.g. aspirin, phenazopyridine, and acetanilide, (viii) non-sulfa antibiotics, e.g. nalidixic acid, nitrofurantoin, isoniazid, dapsone, and furazolidone and, (ix) Henna, a herbal cosmetic has been known to cause haemolytic crisis in G6PDH-deficient infants [28].

The severity of CNSHA associated with G6PDH deficiency is that G6PDH/NADPH pathway is the only source of reduced glutathione in red blood cells (erythrocytes). Inactive G6PDH is unable to synthesize reduced form of NADPH. As a result, under oxidative stress, when all remaining reduced glutathione is consumed, enzymes and other proteins (including hemoglobin) are subsequently damaged by the oxidants, leading to electrolyte imbalance, cross-bonding and protein deposition in the red cell membranes. Damaged red cells are phagocytosed and sequestered in the spleen. The hemoglobin is metabolized to bilirubin, causing jaundice at high concentrations. In addition to jaundice, hemoglobin is excreted directly by the kidney under severe cases, causing acute renal failure [28].

Glucose-6-phosphate Dehydrogenase Over Activation. The role of G6PDH over activation has recently been implicated in the development of 'Syndrome X', also known as 'metabolic syndrome'. Metabolic syndrome is a cluster of conditions that greatly increases the risk of cardiovascular disease (CVD) and type 2 diabetes (T2D). Currently, millions of individuals around the world are affected by this disease. Patients with this disease concurrently show insulin resistance, visceral obesity or belly fat, atherogenic dyslipidemia (comprises a triad of increased blood concentrations of small, dense low-density lipoprotein-LDL, decreased high-density lipoprotein-HDL and increased triglycerides) and hypertension.