A Guide for Future Therapeutics Based upon the Function of Enzymes and Proteins in Human Pathologic Metabolic Processes

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Abstract: The investigation updates information on enzymes and proteins related to their classification, functions, properties, and role in human pathology. Enzymes are any of a group of complex or conjugated proteins that are produced by living cells and act as catalysts in specific biochemical reactions. The three types of enzymes, metabolic, digestive and food-based, play key roles in the treatment of all the major sources of morbidity and mortality including cancer, dementia, diabetes, cardiac disease, and obesity. The ability to accurately target metabolic pathways and pathologic pathways allow adaptations by changing the expression of specific enzymes implicated in the pathogenesis or prevention of diseases. This overview provides a summary to guide the development of enzyme-based therapeutics. The changes of expression and activity of lipid metabolising enzymes are directly regulated by oncogenic signals. Hyper activation of the Poly (ADP-ribose) polymerase [PARP] pathway may be exploited to selectively kill cancer cells. Amyloid beta (Aβ) peptides play a major role in the pathogenesis of Alzheimer's disease (AD). Sphingolipid metabolites play important roles in the regulation of glucose metabolism. In diabetes and insulin resistance, sphingosine kinase 1 (SPK1) is the key enzyme in the sphingolipid metabolic pathway. SPK1 gene therapy may represent a novel approach to wound healing related to diabetes. Several P450s enzymes modulate important steps in the pathogenesis of ischemic heart disease (IHD). The homologous sirtuin (Sirt) family of proteins have beneficial effects in metabolism and aging-related diseases in mammalian systems. These proteins play an important role in maintaining neuronal health during aging.

Keywords: Mitochondria, metastasis, obesity, caloric restriction, cardiovascular disease.

INTRODUCTION

Enzymes are thermo-labile organic catalysts, proteinaceous in nature and produced by living organisms but are not dependent upon the living organisms for their action. A catalyst is a substance that speeds up a chemical (metabolic) reaction. The catalyst itself is not used up as a result of its actions. They act like the engine of a motor and are defined as any of a group of complex or conjugated proteins that are produced by living cells and act as catalysts in specific biochemical reactions [1]. There are two types of enzymes: simple – composed of alpha-amino acids, e.g., pepsin, trypsin and urease, and conjugated – which consist of protein plus non-protein, which does the work. The protein part is called as apo enzyme. The non-protein component is known as the co-factor, which is either a prosthetic group containing a metal, e.g., zinc (Zn) which is the activator or a coenzyme, e.g., dismutase peroxidase which requires vitamin E as coenzyme. It should be stated that the separated protein and non-protein components are inactive. The prosthetic group represents a firmly attached coenzyme such as iron protoporphyrin and the term activator is reserved for metals which are non-reducing agents [1].

They catalyze chemical reactions of other substances without them being destroyed or altered upon completion of the reactions [2]. In order for biochemical reactions to take place enzymes are necessary. Enzymes speed up the chemical reactions of the cell and are never consumed or used up during the reaction. They can do their job over and over again [3]. Proteins that function as biological catalysts are called enzymes. They are composed of carbon (C), hydrogen (H), oxygen (O) and nitrogen (N). Sulphur (S) may also be present [4]. Low contamination, low temperature and fast metabolism are only possible with enzymes.

The objective of this investigation is to report up-to-date information on enzymes and proteins related to their classification, functions, properties, and role in human organs and diseases.

CLASSIFICATION AND FUNCTIONS OF ENZYMES

There are three general classifications of enzymes: 1 - Metabolic enzyme**s** are those, which direct our body. They act as a catalyst to the growth of bone, tissue rebuilding, regulating metabolism, speaking, breathing, reproduction, hearing and any muscle

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contraction. These metabolic enzymes are found naturally in our body's biochemistry. However, as we grow older, metabolic enzyme activity weakens. 2 - Digestive enzymes which are necessary for food digestion. These enzymes break down proteins, carbohydrates and fats in food. They are also responsible for retrieval, assimilation, metabolism and absorption of nutrients, vitamins and minerals. 3 - Food enzymes come from food we eat and help start the digestive process. All raw foods, namely fruits, vegetables, seeds, nuts, some concentrated foods of high importance, especially seeds, are the main resource for food enzymes. Raw meat and fish also contain enzymes. Meat or fish has to be cooked very well because of a number of pathogens and parasites. "Raw food" implies that the food is not animal. Details on classification and numbering of enzymes and examples can be found elsewhere [5].

Most enzymes are formed of protein, although there is a subset of proteins formed from RNA known as ribozymes [6]. The general mechanism by which enzymes work is by reducing the energy required to start the reaction, otherwise known as the activation energy. Enzymes have extremely varied uses: ethanol production, forensic science, and polymerase chain reaction amongst them. Biologists aren't the only people interested in enzymes, however. These diverse molecules form a huge part of industries such as brewing and pharmaceuticals; the use of enzymes alone in the brewing process of Guinness is worth over a billion euro to the Irish economy [6].

PROPERTIES OF ENZYMES

Enzymes are affected by heat, temperature, pH, and pressure and are denatured by excess heat or cold, i.e. their active site becomes permanently warped, and thus the enzyme is unable to form an enzyme substrate complex. This is what happens when you fry an egg. The egg white, a type of protein - not an enzyme, is denatured. Enzymes are created in cells but are capable of functioning outside of the cell. This allows the enzymes to be immobilised, without killing them. The rate at which enzymes can conduct reaction is dependent upon the pH of where the reaction is taking place, e. g. pepsin in the stomach has an optimum pH of about 2. Whereas salivary amylase has an optimum pH of about 7.4 [7].

Enzymes are capable of working in reverse, this acts as a cut off point for the amount of product being produced. If there are excess reactants, the reaction will keep going and be reversed, so that there is no overload or buildup of the product. They are all proteins and biological catalysts, which is one reason why humans need protein in their diet. They speed up a reaction without being used up; this means they can be used over and over again and a small amount of enzyme can affect the change of a large amount of chemical. Enzymes are specific, that is they control or catalyze only one reaction. For example, maltase only acts on maltose and sucrase on sucrose etc. [7].

ROLE OF ENZYMES AND PROTEINS IN IMPORTANT HUMAN BODY ORGANS AND DISEASES

In this section, factors including body organs, metabolic processes and diseases in humans, as influenced by various enzymes and proteins will be discussed. Table **1** provides a summary of these enzymes.

a. Cancer

Although cancer has historically been viewed as a disorder of proliferation, recent evidence has suggested that it should also be considered a metabolic disease. Growing tumors rewire their metabolic programs to meet and even exceed the bioenergetic and biosynthetic demands of continuous cell growth [8]. The metabolic profile observed in cancer cells often includes increased consumption of glucose and glutamine, increased glycolysis, changes in the use of metabolic enzyme isoforms, and increased secretion of lactate. Oncogenes and tumor suppressors have been discovered to have roles in cancer-associated changes in metabolism as well. The metabolic profile of tumor cells has been suggested to reflect the rapid proliferative rate. [8]. Cancer is among the leading causes of death in Canada [9]. In 2011, 29.9% of all death in Canada were due to cancer, while heart related diseases were 19.7% [9]. Cancer is a disease involving unregulated cell growth and grows uncontrollably forming malignant tumors, and invades nearby parts of the body. There are over 200 different known cancers that afflict humans [10]. In 2007, cancer caused about 13% of all human deaths worldwide (7.9 million) [10].

Cancer is one of the most life-threatening diseases with many forms still regarded as incurable. The conventional cancer treatments have unwanted side effects such as the death of normal cells [11]. A therapy that can accurately target and effectively kill tumor cells could address the inadequacies of the

Table 1: Summary of Key Enzymes for Different Disease Processes along with the Potential Therapeutic Targets

Diseases	Key Enzymes	Potential Therapeutic Targets
Cancer	Prostate-specific membrane antigen [PSMA] Prostate Cancer	Intravenous administration of BAY 1075553
	Pyruvate kinase M2 (PKM2) Multiple cancers	Ligand-induced protein kinase activity (PKM2-SAICAR)
	Matrix metalloproteinase (MMPs) overexpression Tumor cell disruption	MMPs and MMP-9 as drug targets
	Receptor tyrosine kinase (RTK) activation Esophageal cancer	RTK suppression
	Hydroxysterol sulfotransferase 2B1b (SULT2B1b) Apoptosis	Oxysterol sulfation as a regulatory signaling pathway
	PARP pathway activation (tankyrase) Telomere elongation	Inhibition of PARP pathway
	Overexpression of activation-induced cytidine deaminase (AID) and protein kinase c iota (PKCi) protein Gastric carcinoma	Reduced expression of AID and PKCi
Dementia	Polyunsaturated fatty acid peroxidization Alzheimer's (AD) and Parkinson's (PD)	Lipid hydro peroxide-and aldehyde modified protein reduction
	Extra-and intra-cellular neurotoxic amyloid β (Aβ) peptides AD	Intracellular hyperphosphorylated tau protein reduction
	Mitochondrial dysfunction AD and PD	ID 1201 having anti-amyloidogenic effect via the activation of the P13K/Akt pathway; inhibition of ERK
	Impaired proteasomal / lysosomal degradation PD	Alpha-sinuclein reduction; resveratrol
Diabetes and Insulin Resistance	Sphingolipid metabolites	Sphingosine kinase (SPK1) gene therapy
	Angiotensin-converting enzyme 2 (ACE 2)	ACE inhibitors
	Zinc (Zn) dependent glucose metabolism	Zn supplementation by preserving the glucose-metabolism-related pathways via preventing upregulation of Akt negative regulators
Heart related disorders, and cardio vascular diseases	NADPH oxidase (NOx) family, especially the NOx 2 and 4 isoforms	Targeting individual NOx isoforms and the unfolded protein response signaling pathway
	Akt activity	Sirturin-dependent regulation of Akt signaling to control cellular processes
	Histone deacetylase enzymes	Ezetimibe
	Deficiency of lysosomal enzyme alpha-galactosidase A	Replacement with recombinant human alpha-galactosidase A
	P450 enzyme-CYP3A4	Amlodipine use
Obesity, ageing and caloric restriction	Increased expression of pro-inflammatory adipokines, diminished expression of anti-inflammatory adipokines, insulin resistance, leptin levels	Enzyme Sir2 silencing genes HST1 overexpression restores transcriptional silencing and contribute to proper cell cycle progression
	NAD dependent deacetylases	Mechanism of caloric restriction promoting longevity.

available therapies. Atmospheric gas plasmas (AGP) have been shown to exploit tumor-specific genetic defects and a recent trial in mice has confirmed its antitumor effects [11]. The mechanism by which the AGP act on tumor cells but not normal cells is not fully understood. It appears that pro-oncogene or tumor

suppressor-dependent regulation of antioxidant/or ROS signaling pathways may be involved in AGP-induced cancer cell death. The toxic effects of ROS are mitigated by normal cells by adjustment of their metabolic pathways [11].

Cancer cells may undergo metabolic adaptations that support their growth as well as drug resistance properties. It has been indicated [12] that the metabolic defects introduced by siRNA silencing of metabolic enzymes transketolase (TKT) or adenylate kinase (AK2) may be compensated by alternative feedback metabolic mechanisms, suggesting that cancer cells may overcome single defective pathways through secondary metabolic network adaptations. The highly robust nature of oral cancer cell metabolism implies that a systematic medical approach targeting multiple metabolic pathways may be needed to accomplish the continued improvement of cancer treatment [12].

Prostate-specific membrane antigen (PSMA) is a transmembrane protein overexpressed in prostate cancer and is therefore being explored as a biomarker for diagnosing and staging of the disease [13]. BAY 1075553 was identified as a promising positron emission tomography (PET) tracer for PSMA-positive prostate tumors in preclinical studies. BAY 1075553 can be produced using a robust, direct radio-synthesis procedure. Pharmacokinetic, toxicology and safety pharmacology studies support the application of BAY 1075553 in a first-in-man micro dose study with single intravenous administration [13].

Cancer cells metabolically adapt to undergo cellular proliferation. Lipids, besides their well-known role as energy storage, represent the major building blocks for the synthesis of neo-generated membranes. There is increasing evidence that cancer cells show specific alterations in different aspects of lipid metabolism. The changes of expression and activity of lipid metabolising enzymes are directly regulated by the activity of oncogenic signals [14].

Accelerated cholesterol and lipid metabolism are the hallmarks of cancer and contribute to malignant transformation due to the obligatory requirement for cholesterol for the function of eukaryotic membranes. To build new membranes and maintain active signaling, cancer cells depend on high intensity of enzyme-catalyzed endogenous cholesterol biosynthesis and uptake of lipid particles [15]. These recently discovered "moonlighting" activities of the cholesterol pathway genes and metabolites expand our understanding of the uniquely conserved roles these sterol molecules play in the regulation of cellular proliferation and in cancer [15].

Abnormal metabolism and sustained proliferation are also hallmarks of cancer [16]**.** Pyruvate kinase M2 (PKM2) is a metabolic enzyme that plays important roles in both processes. In particular, PKM2-SAICAR phosphorylates activates extracellular signal-regulated kinases (Erk1/2), which in turn sensitizes PKM2 for succinylaminoimidazolecarboxamide ribose-5′-phosphate (SAICAR) binding through phosphorylation. Additionally, PKM2-SAICAR was necessary to induce sustained Erk1/2 activation and mitogen-induced cell proliferation. Thus, the ligand-induced protein kinase activity from PKM2 is a mechanism that directly couples cell proliferation with intracellular metabolic status [16].

Matrix metalloproteinases (MMPs) are endopeptidases with the ability to degrade extracellular matrix proteins. In healthy individual tissue disruption is prevented by precise regulation of MMPs; however, in cancer a number of MMPs are overexpressed causing tissue disruption and making tumor cells capable of invasion and metastasis. Therefore, MMPs, and MMP-9 in particular, are reliable candidates for diagnostic biomarker and drug target and further functional analyses have to be performed in order to confirm their role in breast cancer [17].

A better understanding of the differences between signaling pathways present in epithelial cell lines may contribute to reveal the processes underlying the Oral Squamous Cell Carcinoma (OSCC) which is an important cancer disease affecting thousands of people worldwide [18]. Oral cancer disease represents a significant fraction of all human cancer types and its poor early diagnosis contributes to reduced individual survival rate. The identification of proteins modulated in tumorigenic cells and its post-translational modifications may improve our understanding of tumor development in epithelial cells [18].

Esophageal adenocarcinoma (EAC) is the eighth most common cancer worldwide, and approximately 15% of patients survive 5 years [19]. Reflux disease (GERD) and Barrett's esophagus (BE) are major risk factors for the development of EAC, and epidemiologic studies highlight a strong association with obesity. The immune, inflammatory and intracellular signaling changes resulting from chronic inflammation of the esophageal squamous epithelium are increasingly well

characterized. In GERD and BE, an essential role for T-cells in the initiation of inflammation in the esophagus has been identified, and a balance between T-cell responses and phenotype may play an important role in disease progression [19]. Obesity is a chronic low-grade inflammatory state, fueled by adipose tissue derived- inflammatory mediators such as IL-6, TNF-α and leptin, representing a novel area for targeted research. Additionally, reactive oxygen species (ROS) and receptor tyrosine kinase (RTK) activation may drive progression from esophagitis to EAC, and downstream signaling pathways employed by these molecules may be important [19].

Gastric adenocarcinoma is one of the most common malignant tumors and the leading cause of malignancy-related death worldwide. Studies have indicated overexpression of activation-induced cytidine deaminase (AID) and protein kinase c iota (PKCi) proteins showing involvement in the regulation of carcinogenesis [20]. The expression of AID and PKCi was found in patients with gastric adenocarcinoma and was significantly correlated with these proteins [20]. In addition, PKCi expression was significantly correlated with clinicopathological findings such as a lymph node metastasis, and venous and lymphatic invasion ($p < 0.05$). Furthermore, AID expression was significantly correlated with PKCi and mutated p53 protein expression in gastric adenocarcinoma ($p < 0.05$). High AID and PKCi expressions were significantly correlated with poorly-differentiated gastric adenocarcinoma [20].

Hepatocellular carcinoma (HCC) is often diagnosed at an advanced stage, when it is not amenable for aggressive therapies such as surgical resection or liver transplantation. Current therapeutic options achieve clinical responses in only a small percentage of cases [21]. Altered lipid metabolism has recently been linked to HCC pathogenesis. Increased stem cell dysfunction (SCD) expression plays an important role in HCC development and resistance to chemotherapy-induced apoptosis, and this is in part mediated by phosphatidylinositol 3 kinase/c-Jun N-terminal kinases activation [21].

In an effort to arrest DNA synthesis in leukemia cells, both pathways of synthesis must be inhibited. A specific enzyme, deoxycytidine kinase (dCK), is found in both of these pathways. DI-39 is a new high-affinity small-molecule inhibitor of dCK and offers a new pharmacological strategy for stopping cancer cell replication in leukemia [22]. An alternative method of stopping cancer cells from dividing is to take advantage of the concept of replication stress. Replication stress arises from a nucleotide deficiency and results in damage to replicating DNA. Interrupting enzymes that are essential to nucleoside production such as thymidine kinase (TK1) and dCK may induce replication stress by creating a shortage of nucleosides during DNA replication and be lethal to some cancer cells [23].

Intracellular lipid accumulation, inflammatory responses, and subsequent apoptosis are the major pathogenic events of metabolic disorders, including atherosclerosis and non-alcoholic fatty liver diseases [24]. Recently, a novel regulatory oxysterol, 5-cholesten-3b, 25-diol 3-sulfate (25HC3S), was identified, and hydroxysterol sulfotransferase 2B1b (SULT2B1b) were elucidated as the key enzyme for its biosynthesis from 25-hydroxycholesterol (25HC) via oxysterol sulfation. It was proposed that oxysterol sulfation functions as a regulatory signaling pathway [24].

The characteristic of all cancer cells, which systematically reprogram tissue-specific gene expression and activate silent genes, can be exploited to develop new anticancer strategies aiming at the detection of malignant states, the prediction of their evolution and drug sensitivity and the discovery of new therapeutic approaches [25]. A major difficulty in the treatment of cancers is the poor response of many tumors to pharmacological routines [26]. This situation can be accounted for by the existence of a variety of complex mechanisms of chemo resistance (MOCs), leading to reduced intracellular concentrations of active agents, changes in the molecular targets of drugs, enhanced repair of drug-induced modifications in macromolecules, stimulation of anti-apoptotic mechanisms and inhibition of pro-apoptotic mechanism. A more attainable goal in this area of pharmacological enquiry has been suggested as the identification of proteomic profiles that will permit oncologists to accurately predict a lack of response to a given regimen, which would be useful for adapting treatment to the personal situation of each patient [26].

Chemoprevention has been a pivotal and effective strategy during the skin cancer treatment [27]. Using human skin normal and tumor samples, it has been demonstrated that both the expression and activity levels of pyruvate kinase M2 (PKM2) were higher in skin tumor tissues than in normal tissues, suggesting that PKM2, one of the important metabolic enzyme, might serve as a target for skin cancer prevention

and/or therapy. These results suggest that shikonin bears chemo preventive potential for human skin cancers in which PKM2 is upregulated which might be mediated by inhibiting oncogenic activation, PKM2 activation, and mitochondrial dysfunction [27].

Metastasis and drug resistance are the major limitations in the survival and management of patient particularly with colon cancer [28]. In the later study, c-Yes and Yap were identified as potential molecular targets to eradicate quiescent cancer cells and dormant micro metastases during 5FU chemotherapy and resistance and as predictive survival markers for colon cancer [28]. Hyperactivation of the PARP pathway may also be exploited to selectively kill cancer cells. Other PARP forms, including tankyrase 1 (PARP 5a), which plays an important role in enhancing telomere elongation by telomerase, have been found to be potential targets in cancer therapy. The PARP pathway and its inhibition thus offers a number of opportunities for therapeutic intervention in both cancer and other disease states [29].

b. Dementia

Dementia includes a chronic or persistent disorder of the mental processes caused by brain disease or injury marked by memory disorders, personality changes and impaired reasoning. Dementia is not an illness or disease in itself, but it is a broad term which is used to describe a range of signs and symptoms that occur when brain is affected by certain diseases and conditions. Examples include Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD).

Polyunsaturated fatty acid (PUFA) is easily peroxidized by free radicals and enzymes. When this occurs, it results in the compromised integrity of cellular membranes and leads to lipid hydroperoxide as a major reaction product, which is decomposed into aldehyde [30]. The lipid hydroperoxide- and aldehyde-modified proteins have been immunohistochemically detected in diverse pathological situations such as atherosclerosis, AD, PD, chemical material-induced liver injury and renal tubular injury in humans and experimental animals. It suggests that the production of lipid hydroperoxide and aldehyde-modified proteins is closely associated with the pathogenesis of these diseases in humans and experimental animals [30].

Alzheimer's disease is the most common cause of dementia in the elderly. With an increasing longevity and the absence of a cure, AD has become not only a major health problem but also a heavy social and economic burden worldwide [31]. In addition to the presence of abundant intra- and extra-cellular neurotoxic amyloid β (Aβ) peptides, which form the amyloid plaques, and intracellular hyperphosphorylated tau protein, the main component of neurofibrillary tangles, consistent evidence shows that the AD brain is characterized by extensive neuroinflammatory processes. This indicates that this protein as pleiotropic contributes to the development of the full spectrum of the AD-like phenotype in mouse models of the disease, making it a viable therapeutic target for the treatment of AD in humans [31].

Amyloid beta (Aβ) peptides, which are generated from amyloid precursor protein (APP), are thought to play a major role in the pathogenesis of AD [32]. The anti-amyloidogenic effects of the ethanolic extract of Meliae Fructus (ID1201)was investigated using human embryonic kidney 293 cells with stably expressed human wild-type or Swedish mutant APP695 and β-secretase 1. Results of the later study also indicated that ID1201 possesses anti-amyloidogenic effects via the activation of the PI3K/Akt pathway, suggesting that it is a potential therapeutic agent for AD [32].

Mitochondrial dysfunction is an early pathological feature of AD. The underlying mechanisms and strategies to repair it remain unclear. Studies have demonstrated that the direct consequences and potential mechanisms of mitochondrial functional defects are associated with abnormal mitochondrial dynamics in AD [33]. The protective effect of inhibition of extracellular signal-regulated kinase (ERK) signaling on maintenance of normal mitochondrial structure and function holds promise as a potential novel therapeutic strategy for AD [33].

The public health burden of metabolic syndrome (MetS), a multiplex risk factor that arises from insulin resistance accompanying abnormal adipose conditions and AD, the most common form of dementia, continues to expand [34]. Aberrant sphingolipid profiles have been observed in human AD brains, and accumulated evidence has demonstrated that changes in membrane properties induced by defective sphingolipid metabolism impair generation and degradation of amyloid-β peptide (Aβ), a pathogenic agent of AD [34].

The clinical recognition of a form of dementia closely resembling AD dates from around 1800 [35]. Spontaneous reinnervation, N-acetyl cysteine

administration and tyrosine supplementation may attenuate the early stages of Fischer-Alzheimer disease [F-AD] development [35]. The role of analgesics derived from coal-tar in the spread of the pandemic is traced in terms of the introduction of phenacetin (PN) in 1887. Its nephrotoxicity; the observation of lesions characteristic of the disease by Fischer and Alzheimer and the discovery of paracetamol (PA) as the major metabolite of PN. This links kidney injury and dementia with high PN usage; and the failure of PN replacement by PA to halt and reverse the exponential, inexorable rise in the incidence of Alzheimer-type dementia. Fischer-Alzheimer disease is primarily a man-made condition with PA as its principal risk factor [35].

Autism has similarity to AD in that, e.g., wandering behavior seen in individuals with autism can also be manifested in seniors who have dementia. Autism spectrum disorder (ASD) is the term used for a diverse group of developmental conditions that affect a person's ability to relate to and communicate with others [36]. There is widespread hope that the discovery of valid biomarkers for autism will both reveal the causes of autism and enable earlier and more targeted methods for diagnosis and intervention. However, growing enthusiasm about recent advances in this area of autism research needs to be tempered by an awareness of the major scientific challenges and the important social and ethical concerns arising from the development of biomarkers and their clinical application [36]. The central dopaminergic system is strongly implicated in ASD pathogenesis. Genes encoding various elements of this system including dopamine receptors, the dopamine transporter or enzymes of synthesis and catabolism have been linked to ASD. It emphasizes the important role of the dopaminergic system in ASD, and implicate several cellular signaling processes in its pathogenesis [37].

Drug candidates directed against amyloid-β (Aβ) are mainstream in AD drug development. Active and passive Aβ immunotherapy is the principle that has come furthest, both in number and in the stage of clinical trials. Last, downstream biomarker evidence (CSF tau proteins and MRI volumetric) that the drug ameliorates neurodegeneration will, together with beneficial clinical effects on cognition and functioning, be essential for labeling an anti-Aβ drug as disease modifying [38].

Elevated concentration of homocysteine (Hcy) in human tissues, resulting either from mutations in genes enconding Hcy-metabolizing enzymes, or from deficiencies of folic acid has recognized cytotoxic effect. Even a mild Hcy level increase is a risk factor for cardiovascular diseases (CVDs) and stroke in humans and also a risk factor for neurodegenerative disorders, such as dementia, or AD. However, it is not yet clear whether homocysteine is a marker, or a causative agent [39].

Parkinson's disease is a multicentred neurodegenerative disorder characterised by the accumulation and aggregation of alpha-synuclein (α-syn) in several parts of the central nervous system (CNS). However, it is well established that PD can generate symptoms of constipation and other gastrointestinal problems and α-syn containing lesions have been identified in intestinal nerve cells. It has been shown [40] that α-syn can be taken up and accumulate in primary human foetal enteric neurons from the gastrointestinal tract and can be transferred between foetal enteric neurons. Impaired proteasomal/lysosomal degradation can promote the uptake and accumulation of α-syn in enteric neurons. Enteric neurons exposed to α-syn can also lead to impaired mitochondrial complex I activity, reduced mitochondrial function, and NAD+ depletion culminating in cell death via energy restriction [40].

Mitochondrial dysfunction and oxidative stress occur in PD, but the molecular mechanisms controlling these events are not completely understood. The functional impact of resveratrol treatment encompassed an increase of complex I and citrate synthase activities, basal oxygen consumption, and mitochondrial ATP production and a decrease in lactate content, thus supporting a switch from glycolytic to oxidative metabolism [41]. Moreover, resveratrol treatment caused an enhanced macro-autophagic flux through activation of an LC3-independent pathway. Thus in early-onset PD fibroblasts, resveratrol may have potential clinical application in selected cases of PD-affected patients [41].

c. Diabetes and Insulin Resistance

Diabetes is one of the most prevalent human metabolic diseases. Wound healing in diabetes is frequently impaired and treatment remains challenging [42]. Sphingolipid metabolites play important roles in the regulation of glucose metabolism. Sphingosine kinase 1 (SPK1) is the key enzyme in the sphingolipid metabolic pathway. SPK1 gene therapy may represent a novel approach to cutaneous wound healing [42].

Oxidative stress induced by hyperglycemia is a key factor in the pathogenesis of diabetes complications. Glutaredoxin 1(Grx1) is a cytosolic redox protein that catalyzes GSH-dependent thiol redox reactions and reversible protein S-glutathionylation [43]. This indicated an extracellular function of plasma Grx in blood glucose metabolism. Thus, Grx may be a marker of increased oxidative stress during hyperglycemia in healthy subjects and may be a risk marker of progression toward diabetes onset [43].

Liver gluconeogenesis is essential to provide energy to glycolytic tissues during fasting periods. However, aberrant up-regulation of this metabolic pathway contributes to the progression of glucose intolerance in individuals with diabetes [44]. Liver and muscle glycogen content is reduced in diabetic patients but there is no information on the effect of diabetes on the glycogen content in the retinal pigment epithelium (RPE) [45]. It was concluded that glycogen storage is increased in the RPE of diabetic patients, and it is associated with an increase in glycogen synthase (GS) activity.

Insulin resistance is a major underlying mechanism responsible for the 'metabolic syndrome', which is also known as insulin resistance syndrome [46]. The incidence of metabolic syndrome is increasing at an alarming rate, becoming a major public and clinical problem worldwide. Metabolic syndrome is represented by a group of interrelated disorders, including obesity, hyperglycemia, hyperlipidemia, and hypertension [46]. It is also a significant risk factor for the cardio vascular disease (CVD) and increased morbidity and mortality.

Angiotensin-converting enzyme 2 (ACE2) is located in several tissues and is highly expressed in renal proximal tubules, where it degrades the vasoconstrictor angiotensin II (ANG II) to ANG-(1-7). Accumulating evidence supports protective roles of ACE2 in several disease states, including diabetic nephropathy [47]. The results of this study showed an association between hyperglycemia, CV risk factors, and increased shedding of urinary ACE2 in diabetic Akita mice. Urinary ACE2 could be used as a biomarker for diabetic nephropathy and as an index of intrarenal ACE2 status [47].

Human epidemiological and animal studies have shown the beneficial therapeutic effect of Zn supplementation on mitigating diabetic nephropathy. However, the mechanism by which Zn protects the kidney from diabetes remains unknown [48]. Furthermore, Zn-stimulated changes in glucose metabolism mediated by Akt were actually found to be metallothionein dependent, but not Akt2 dependent. This suggests that the therapeutic effects of Zn in diabetic nephropathy are mediated, in part, by the preservation of glucose-metabolism-related pathways via the prevention of diabetes-induced upregulation of Akt negative regulators [48]. Given that Zn deficiency is very common in diabetics, this finding implies that regularly monitoring Zn levels in diabetic patients, as well as supplementing if low, is important in mitigating the development of diabetic nephropathy [48].

d. Heart-Related Disorders, Cardiovascular Disease [CVD] and Statins

The P450 enzymes (P450s) mediate the biotransformation of several drugs, steroid hormones, eicosanoids, cholesterol, vitamins, fatty acids and bile acids, many of which affect CV homeostasis. Experimental studies have demonstrated that several P450s modulate important steps in the pathogenesis of ischemic heart disease (IHD) [49]. It might be stated that insulin resistance is also a significant risk factor for CVD and increased morbidity and mortality.

Reactive oxygen species (ROS) are produced during normal endoplasmic reticulum (ER) metabolism. There is accumulating evidence showing that under stress conditions such as ER stress, ROS production is increased via enzymes of the NADPH oxidase (NOx) family, especially via the NOx2 and NOx4 isoforms, which are involved in the regulation of blood pressure [50]. Hypertension is a major contributor to CVD and renal disease, and it has a complex pathophysiology involving the heart, kidney, brain, vessels, and immune system. The development of specific approaches that target individual NOx isoforms and the unfolded protein response (UPR) signaling pathway may be important for the achievement of therapeutic efficacy in hypertension [50].

Cardiac hypertrophy is a multifactorial disease characterized by multiple molecular alterations. One of these alterations is change in the activity of Akt, which plays a central role in regulating a variety of cellular processes ranging from cell survival to aging [51]. Akt activation is mainly achieved by its binding to phosphatidylinositol (3, 4, 5)-triphosphate. Implications of sirtuin-dependent regulation of Akt signaling in the control of major cellular processes such as cellular growth, angiogenesis, apoptosis, autophagy, and aging, which are involved in the initiation and

progression of several diseases have been described in detail [51].

Vascular diseases, including atherosclerosis, angioplasty-induced restenosis, vessel graft arteriosclerosis and hypertension-related stenosis, remain the most prevalent cause of death in the developed world. The aetiology of vascular diseases is multifactorial with both genetic and environmental factors [52]. Recently, some of the most promising research identifies the epigenetic modification of the genome to play a major role in the disease development, linking the environmental insults with gene regulation [52]. Recent studies demonstrated that histone deacetylase (HDAC) enzymes are crucial in endothelial integrity, smooth muscle proliferation and in the formation of arteriosclerosis in animal models. This provides a new approach for the treatment of vascular disease using the agents that influence the epigenetic process in vascular cells [52]. Inflammation plays a crucial role in atherosclerosis. Monocytes/ macrophages are involved in the inflammatory process during atherogenesis [53]. These results provide direct evidence for the potential application of ezetimibe in treating high cholesterol and in the prevention and treatment of inflammatory diseases [53].

Circulating levels of cholesterol precursors in the body have proven their value over the years as indicators of in-vivo cholesterol synthesis [54]. However, there is growing interest in their potential as markers of various disease states. Beyond their accepted application as markers of cholesterol synthesis, cholesterol precursors have potential both as disease indicators, and for providing deeper insights into the disease process [54]. The atherosclerotic process is driven by elevated Low-density lipoprotein (LDL)-cholesterol in combination with enhanced inflammatory responses [55]. Several mediators participate in this complex inflammatory network including members of the tumor necrosis factor (TNF) (receptor) superfamily. These findings may implicate a pathogenic role for inflammation and TNF related molecules in familial hypercholesterolemia (FH), and these findings suggest the possibility that novel treatment modalities beyond that of statins and lipid lowering drugs may be useful in FH subjects [55].

Iron (Fe) is an essential mineral in many proteins and enzymes in human physiology, with limited means of Fe elimination to maintain Fe balance. Iron accrual incurs various pathological mechanisms linked to CVD [56]. In atherosclerosis, Fe catalyzes the creation of oxygen free radicals that contribute to lipid modification, which is essential to atheroma formation. The role of Fe in atherosclerosis with considerable implications for novel diagnostic and therapeutic approaches has been summarized [56].

Fabry's is a progressive, destructive and life threatening disease which reduces significantly the life expectancy of the affected individual [57]. It is a genetic disorder of X-linked inheritance caused by deficiency of lysosomal enzyme alpha-galactosidase A resulting in progressive accumulation of glycosphingolipids within different body cells. Specific therapy for Fabry's disease is enzyme replacement with recombinant human alpha-galactosidase A. If started early it has a promising role in renal and cardiac disease however beneficial role is not yet defined in CNS involvement [57].

Amlodipine is in a group of drugs called calcium channel blockers. It relaxes blood vessels and improves blood flow and is used to treat hypertension or angina and other conditions caused by coronary disease. It is commonly prescribed calcium channel blocker for the treatment of hypertension and ischemic heart disease [58]. The drug is slowly cleared in humans primarily via dehydrogenation of its dihydropyridine moiety to a pyridine derivative (M9). Furthermore, metabolism of amlodipine in expressed human P450 enzymes showed that only CYP3A4 had significant activity in amlodipine dehydrogenation. These results indicate that CYP3A4, rather than CYP3A5, plays a key role in metabolic clearance of amlodipine in humans [58].

Statins are the cornerstone of lipid-lowering therapy to reduce the risk of coronary heart disease. Rosuvastatin and pitavastatin are the two recently developed statins with less potential for drug interaction resulting in improved safety profiles [59]. However, drug transporters play a significant role in the disposition of rosuvastatin and pitavastatin and drug interactions may occur through these. Genetic polymorphisms in drug transporters may also affect the pharmacokinetics, drug interactions and/or the lipid-lowering effect of these statins to a different extent [59].

e. Obesity and Caloric Restriction Associated with Ageing

Obesity is a risk factor for various CVD diseases including hypertension, atherosclerosis, and myocardial infarction [60]. Obesity leads to increased expression of

pro-inflammatory adipokines and diminished expression of anti-inflammatory adipokines, resulting in the development of a chronic, low-grade inflammatory state. This adipokine imbalance is thought to be a key event in promoting both systemic metabolic dysfunction and CVD [60].

Obesity is associated with insulin resistance and chronic low-grade inflammation. Insulin has been described to have anti-inflammatory effects in immune cells [61]. Therefore, insulin resistance in immune cells can be expected to have important consequences for immune function. It was shown that freshly isolated monocytes, but not T cells, are insulin-sensitive cells and that this insulin sensitivity of monocytes is not negatively affected by the glucometabolic state of the donor [61]. Obesity is also linked to increased cancer risk. Pathological expansion of adipose tissue impacts adipocyte function and secretion of hormonal factors regulating tissue homeostasis and metabolism [62]. Despite significant advances in understanding Adiponectin functions and signaling mechanisms, its role in cancer remains multifaceted and subject to controversy [62].

Dysregulation of adipose hormones in obesity has been associated with the hastened development of metabolic syndrome and associated chronic disease sequalae, a condition that is a consequence of previous disease or injury, including CVD and type 2 diabetes mellitus [63]. Leptin levels were associated with measures of adiposity but not liver enzymes. Each of these variables, along with blood lipids, may serve as potential future therapeutic targets for the prevention and management of obesity and related comorbidities [63].

Based on the previous research work in rats, mice, monkeys, and other species, it has been found that the lifespan can be extended to 20% or more when strict calorie restricted diets are imposed on the creatures. Discovery at Massachusetts Institute of Technology showed that an enzyme Sir_2 may also be a link between reduced consumption and longevity. Geneticists think that eating less can extend life in remarkable ways, because it slows the rate of genetic accidents that increase with age. At least three Sir2 silencing genes (HST) can function in silencing; HST1 overexpression restores transcriptional silencing to a sir2 mutant and HST3 and HST4 double mutants are defective in telomeric silencing [64]. In addition, HST3 and HST4 together contribute to proper cell cycle progression, radiation resistance, and genomic stability, establishing new connections between silencing and these fundamental cellular processes [64],

When calories are reduced there are fewer food molecules to break down. This frees up more energy carriers to fuel Sir_2 and its gene silencing work. This persistence of genomic silencing may slow ageing-related processes, such as genome instability and inappropriate gene expression. Calorie restriction extends lifespan in a broad range of organisms, from yeasts to mammals [65]. Numerous hypotheses have been proposed to explain this phenomenon, including decreased oxidative damage and altered energy metabolism [65]. The silencer information regulator (Sir) family of proteins has attracted much attention during the past decade due to its prominent role in metabolic homeostasis in mammals [66]. The Sir1- 4 proteins were first discovered in yeast as nicotinamide adenine dinucleotide (NAD+) - dependent deacetylases, which through a gene silencing effect promoted longevity. The subsequent discovery of a homologous sirtuin (Sirt) family of proteins in the mammalian systems soon led to the realization that these molecules have beneficial effects in metabolism- and aging-related diseases [66].

f. Miscellaneous Factors

This section includes a mix of various factors related to metabolic functions where only sporadic information is available.

Saffron, a spice derived from the flower of *Crocus sativus***,** is rich in carotenoids. Two main natural carotenoids of saffron, crocin and crocetin, are responsible for its color. Preclinical studies have shown that dietary intake of these and other carotenoids have potent anti-tumor effects both *in vitro* and *in vivo*, suggesting their potential preventive and/or therapeutic roles in several tissues [67]. The antitumor actions of saffron and its components have been proposed in the inhibitory effect on cellular DNA and RNA synthesis, but not on protein synthesis; the inhibitory effect on free radical chain reactions; the metabolic conversion of naturally occurring carotenoids to retinoids; and the interaction of carotenoids with topoisomerase II, an enzyme involved in cellular DNA-protein interaction [67].

The beneficial effects of vitamin D_3 are exerted through 1α, 25-dihydroxyvitamin D3 [1α, 25(OH) 2 D 3], the dihydroxy metabolite of vitamin D3. Hepatic and intestinal biotransformation of 1α, 25(OH) 2D3 and

modifiers of metabolic capacity could be important determinants of bioavailability in serum and tissues [68]. Ginsenosides and their aglycones, mainly 20(S)-protopanaxadiol (aPPD) and 20(S)-protopanaxatriol (aPPT), are routinely ingested as health supplements. The results of this study suggest that ginsenosides, specifically aPPD and aPPT, inhibit the CYP3A4-mediated catabolism of active vitamin D3 in human liver and intestine, potentially providing additional vitamin D-related benefits to patients with cancer, neurodegenerative and metabolic diseases [68].

Regulation of angiogenesis is critical for many diseases. Specifically, pathological retinal neovascularization, a major cause of blindness, is suppressed with dietary ω3-long-chain polyunsaturated fatty acids (ω3LCPUFAs) through antiangiogenic metabolites of cyclooxygenase and lipoxygenase [69]. Cytochrome P450 epoxygenases (CYP2C8) also metabolize LCPUFAs, producing bioactive epoxides, which are inactivated by soluble epoxide hydrolase (sEH) to transdihydrodiols. These results suggest that CYP2C ω3LCPUFA, an enzyme that in humans is encoded by the CYP2C9 gene, metabolites promote retinal pathological angiogenesis. CYP2C8 is part of a novel lipid metabolic pathway influencing retinal neovascularization [69].

Stroke is a neurological condition and may cause changes in hepatic drug-metabolizing enzymes. Hepatic CYP2B eukaryotic is involved in the metabolism of a variety of centrally active substances [70]. These results indicated that patients with acute ischemic stroke may have a decreased CYP2B-mediated metabolism of exogenous and endogenous compounds because of the low level of thyroid hormones [70].

Sirturins [SIRT1] are nicotinamide adenine dinucleotide (NAD +) dependent deacylases that have traditionally been linked with caloric restriction and aging in mammals [71]. These proteins also play an important role in maintaining neuronal health during aging. SIRT 1 plays protective roles in several neurodegenerative diseases including AD, PD, and motor neuron diseases, which may relate to its functions in metabolism, stress resistance, and genomic stability. Drugs that activate SIRT1 may offer a promising approach to treat these disorders [71].

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by joint pain, swelling, stiffness, and progressive destruction of the small joints of hands and feet [72]. Treatment of RA has improved over the past decade. With multiple cytokines well-known now to play a role in the pathogenesis of RA, including tumor necrosis factor alpha, interleukin (IL)-1β, and IL-6, many targeted biological treatments against these cytokines have emerged, changing the treatment of this disease. Tocilizumab (TCZ) may be beneficial in the treatment of other autoimmune diseases, spinal disease, CVD, organ transplantation, and malignancies where elevated levels of IL-6 may play a role in the pathogenesis of these diseases [72].

Herbal medicine, especially traditional Chinese medicine and Ayurvedic medicine have played and still play an important role in fighting against various diseases [73]. Emerging clinical studies regarding traditional Chinese medicine have provided convincing evidence for the first time to gain credibility and reputation outside China. The mechanisms underlying synergistic therapeutic actions of herb medicines are: 1. different agents may regulate either the same or different target in various pathways, and therefore cooperate in an agonistic, synergistic way; 2. regulate the enzymes and transporters that are involved in hepatic and intestinal metabolism to improve oral drug bioavailability; 3. overcome the drug resistance mechanisms of microbial and cancer cells; and 4. eliminate the adverse effects and enhance pharmacological potency of agents by "processing" or by drug-drug interaction [73].

CONCLUSIONS

All three types of enzymes, metabolic, digestive and food-based, have an important role in developing therapeutics for major disease groups including cancer, dementia, diabetes, cardiac disease, and obesity. The ability to accurately target metabolic pathways and pathologic pathways allow adaptations by changing the expression of specific enzymes. This overview serves as a summary of the strategies that are employed in the development of enzyme-based therapeutics.

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