Published in final edited form as:
Curr Pharm Biotechnol. 2013 March 1; 14(3): 342–356.

Peroxisome Proliferator Activated Receptor A Ligands as Anticancer Drugs Targeting Mitochondrial Metabolism

Maja Grabacka[1,*], **Malgorzata Pierzchalska**[1], and **Krzysztof Reiss**[2]

[1]Department of Food Biotechnology, Faculty of Food Technology, University of Agriculture, Krakow 30-149, ul. Balicka 122, Poland [2]Neurological Cancer Research, Stanley S. Scott Cancer Center, Department of Medicine, Louisiana State University Health Science Center, 533 Bolivar St. New Orleans, LA, USA

Abstract

Tumor cells show metabolic features distinctive from normal tissues, with characteristically enhanced aerobic glycolysis, glutaminolysis and lipid synthesis. Peroxisome proliferator activated receptor α (PPAR α) is activated by nutrients (fatty acids and their derivatives) and influences these metabolic pathways acting antagonistically to oncogenic Akt and c-Myc. Therefore PPAR α can be regarded as a candidate target molecule in supplementary anticancer pharmacotherapy as well as dietary therapeutic approach. This idea is based on hitting the cancer cell metabolic weak points through PPAR α mediated stimulation of mitochondrial fatty acid oxidation and ketogenesis with simultaneous reduction of glucose and glutamine consumption. PPAR α activity is induced by fasting and its molecular consequences overlap with the effects of calorie restriction and ketogenic diet (CRKD). CRKD induces increase of NAD^+/NADH ratio and drop in ATP/AMP ratio. The first one is the main stimulus for enhanced protein deacetylase SIRT1 activity; the second one activates AMP-dependent protein kinase (AMPK). Both SIRT1 and AMPK exert their major metabolic activities such as fatty acid oxidation and block of glycolysis and protein, nucleotide and fatty acid synthesis through the effector protein peroxisome proliferator activated receptor gamma 1 α coactivator (PGC-1α). PGC-1α cooperates with PPAR α and their activities might contribute to potential anticancer effects of CRKD, which were reported for various brain tumors. Therefore, PPAR α activation can engage molecular interplay among SIRT1, AMPK, and PGC-1α that provides a new, low toxicity dietary approach supplementing traditional anticancer regimen.

Keywords

AMP-dependent protein kinase; calorie restriction; fatty acid oxidation; glutaminolysis; ketogenesis; SIRT1

1. INTRODUCTION

The increasing incidence of cancer and various metabolic diseases such as diabetes, obesity and cardiovascular disease in modern society has become a challenge for public health care in developed countries. The urge to create new approaches to prevent and fight with these

*Address correspondence to this author at the Department of Food Biotechnology, Faculty of Food Technology, University of Agriculture, Krakow 30-149, ul. Balicka 122, Poland; Tel: 0048 12 662 4796, Fax: 0048 12 662 4750; maja.grabacka@ur.krakow.pl.

CONFLICT OF INTEREST The authors declare no conflict of interest.

disorders require a better understanding of their molecular background. Impairment of cellular energetic homeostasis, imbalance of nutrient provision and utilization evoked by genetic and/or environmental conditions are the common observed symptom in these diseases. Simultaneously, extensive research data provide a deeper insight into the role of diet as a direct factor controlling gene expression on transcriptional or epigenetic level.

This review attempts to outline how the peroxisome proliferator-activated receptor α (PPAR α) activation with synthetic agonists or dietary regimens could be applied to target cancer cells through their metabolic weak points with particular emphasis on mitochondrial function. First, the biological role of PPARs and anticancer activities of their natural and synthetic ligands are briefly introduced. Next, the function of two molecular sensors: sirtuins and AMP-dependent protein kinase (AMPK) is discussed in the context of cellular response to nutritional factors, as well as their role in carcinogenesis. The further parts of the paper concentrate on the metabolic characteristics of cancer cells and discuss how calorie restricted ketogenic diet enhanced by PPAR α ligands administration could overthrow their energetic strategies impeding tumor proliferation or progression. This approach might be a safe and effective supplement to the canonical anticancer therapies, including chemo- and radio-therapy.

2. MOLECULAR MECHANISM OF PPARS ACTIONS

PPARs are considered to be the main transcription factors which govern lipid, carbohydrate and aminoacid metabolism. They belong to the nuclear hormone receptor superfamily and share their common structure pattern: DNA binding domain (DBD), ligand binding domain (LBD), amino- and carboxyterminal transactivation domains AF-1 and AF-2 [1, 2]. Upon ligand binding, PPARs associate with the partner receptor: retinoid × receptor (RXR) and this heterodimer induces transcriptional activation of target genes possessing the consensus peroxisome proliferator response element (PPRE) in the promoter region Fig. (1). Transcriptional repression is also possible in the absence of a ligand, when PPARs physically interact and sequester other factors from forming the transcriptional complex or by recruiting histone deacetylases (HDAC) which induce chromatin condensation [3–5]. Transrepression may take place in the presence of a ligand after PPAR sumoylation, when nuclear corepressor NCoR is recruited to the promoter [6, 7]. For the full activity, the cooperation with other nuclear receptors or coactivators like peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is necessary.

PPAR α is mainly responsible for fat mobilization during fasting and activates mitochondrial and peroxisomal fatty acid β- and ω-oxidation and ketogenesis, simultaneously inhibiting glycolysis and fatty acid synthesis. PPAR α is a chief transcription factor that controls expression of genes involved in these processes: fatty acid binding proteins (FATBP), fatty acid transporters (FAT), carnitine palmitoyltransferase I, mitochondrial acyl-CoA dehydrogenase, peroxisomal acyl-CoA oxidase, microsomal cytochrome P450 related enzymes etc. [8–10].

PPAR γ activation is a necessary step leading to adipocyte differentiation and enhances insulin sensitivity. Synthetic PPAR γ ligands from the class of thiazolidinediones are effective antidiabetic drugs. PPAR β together with PPAR α are known to induce oxidative metabolism and mitochondrial fatty oxidation in myocytes in response to exercise and mitochondrial biogenesis in cardiomyocytes [11–13]. All the PPAR isoforms have strong antiinflammatory activity and they exert antitumorigenic effects due to antiproliferative and proapoptotic activites [14–21].

3. NATURAL AND SYNTHETIC PPAR A LIGANDS AND THEIR ANTICANCER ACTIONS

Dietary fatty acids and their derivatives are the most recognizable ligands for all the three PPAR isoforms, which make them nutrient sensors important for coordinating the cellular response to feeding / fasting state [2, 22]. Medium and long chain fatty acids possessing 12 to 20 carbon atoms in the molecule are ubiquitous PPAR α agonists. Particularly, health-promoting effects of dietary n-3 polyunsaturated fatty acids (n-3 PUFA), such as docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA), are attributed to their ability to activate PPAR α. They upregulate the expression of the enzymes that control fatty acid catabolism in PPAR α dependent fashion. The special class of endogenous PPAR α ligands contains arachidonic acid derivatives, the products of lipoxygenases: hydroxyeicosatetraenoic acids (HETE), especially 8(S)-HETE and leukotriene B4 (LTB4) and the products of cytochrome P450 enzymes, namely epoxyeicosatrienoic acids (EETs) and dihydroxyeicosatrienoic acids (DHETs) [23–27]. The chemical nature of these ligands determines existence of so called `metacrine' metabolic signaling loop: the ligands activate the transcription of the enzymes that degrade them: eicosanoids that activate PPAR α are broken down by the products of genes which are the transactivated by the receptor [27, 28]. This negative feedback loop is a part of inflammation resolution process [27, 28].

N-3 PUFA, in contrast to n-6 fatty acids, have been reported to show interesting anticancer properties as chemopreventive agents. They decrease incidence of chemically induced tumors in animal *in vivo* models [29–32] and in epidemiological studies describing the correlation between n-3 PUFA intake and reduced risk of prostate, breast and colorectal cancer development in humans [33].

Synthetic PPAR α agonists, such as the fibrates (fenofibrate, clofibrate, ciprofibrate, bezafibrate, gemfibrozil), are widely applied in clinic as hypolipidemic drugs, since they efficiently reduce plasma triglyceride and low density lipoproteins (LDL) levels and improve blood HDL to LDL ratio. Among other ligands, Wy14,643 (4-chloro-6-(2,3-xylidino)-2-pirymidinylthioacteic acid), is a frequently used as a very potent PPAR α activator. The compounds mentioned above have been reported to induce proliferation of peroxisomes and promote hepatocellular carcinogenesis in mice and rats and therefore were described as `peroxisome proliferators' (PP) and nongenotoxic carcinogens [34]. However, no liver oriented activity of PPAR α has been observed in humans. The species related differences in the susceptibility to peroxisome proliferators have been attributed to the level and activity of oncogenic c-Myc [35]. In PP sensitive rodent species (but not in humans), PPAR α activation represses the microRNA let-7c gene, which controls c-Myc protein levels by destabilizing its mRNA [35]. Upon chronic exposure to PPAR α ligands, mouse liver cells lack let-7c and overexpress c-Myc, which leads to the cellular transformation [35].

In contrast to the effects on murine liver, hypolipidemic drugs including fibrates, have been shown to significantly reduce the risk of death from cancer in patients. Ten year all-cause mortality study carried out on the population of over 7800 French subjects treated with hypolipidemic drugs of fibrate or statin type, revealed that the use of these drugs is associated with a significantly lower total mortality and a significantly reduced probability of death from cancer in the group of subjects treated with fibrate, compared to untreated dyslipidemic group [36]. Some large randomized trials on gemfibrozil and lovastatin reported their potential chemopreventive effects towards melanoma, nevertheless, other studies have not confirmed unequivocally those observations [37–39].

In the numerous cell culture studies, various members of fibrate family were shown to exert interesting anticancer properties. Clofibrate inhibits human ovarian cancer cell proliferation *in vitro* and in mouse xenograft model [40, 41]. Additionally, this drug synergistically enhances clioquinol action against ovarian cancer [42]. Fenofibrate induces apoptosis and slows down the proliferation rate in Ishikawa endometrial cancer cells and in mantle cell lymphoma [43, 44]. Growth arrest and apoptotic response were also noted in glioblastoma cells treated with gemfibrozil [45].

The molecular mechanisms of fenofibrate anticancer activity have been investigated by our group. First, we observed highly encouraging effects of orally administered fenofibrate in Syrian hamsters bearing subcutaneous Bomirski Hamster Melanoma (BHM) tumors. The treated animals developed significantly fewer metastatic foci in lungs and these secondary tumors were much smaller in size comparing to the control group [18]. Hamsters are a convenient model to study PPAR α activity because, unlike mice and rats, they are similar to humans in their response to fibrates or other peroxisome proliferators and do not show the signs of hepatocarcinogenesis.

In order to shed the light on the background of this potential antimetastatic phenomenon, we carried on cell culture experiments with B16 F10 melanoma cell line, which showed that fenofibrate, in a PPAR α dependent fashion, strongly inhibited cell migration, proliferation in anchorage-dependent and –independent conditions, and sensitized the cells to the proapoptotic activity of staurosporine. All these effects were mediated by the repression of Akt phosphorylation [20].

Next, we asked the question, whether other tumor cell lines which share the common neuroectodermal origin with melanoma, are sensitive to fenofibrate as well. Indeed, various medulloblastoma and glioma cell lines responded to fenofibrate with growth arrest, increased apoptosis and repressed motility which correlated with the attenuation of IGF-1receptor (IGF-1R) signaling [21, 46]. Interestingly, the experiments on glioma cells demonstrated that the treatment with a reactive oxygen species (ROS) scavenger N-acetylcysteine restores cell motility, suggesting that the mechanism of fenofibrate action involves, at least in part, PPAR α-dependent ROS accumulation [21]. These new findings encourage the idea of using fenofibrate as a candidate drug for supportive therapies against growth factor mediated cell proliferation, survival and migration events in tumors derived from neuroectodermal tissues.

Fibrate drugs are responsible for a rare but severe side effect in the form of rhabdomyolysis [47]. This fact supported the idea of using these compounds against rhabdomyosarcoma [48]. Indeed, the specific toxicity toward human rhabdomyosarcoma cells have been observed, while no harm to microvascular endothelial cells was detected [48]. Although, vascular cells viability was not affected by fenofibrate, one of its anticancer activities seems to operate through blocking the tumor angiogenesis [49, 50]. A very interesting report by Kaipainen and coworkers suggested an unexpected role of PPAR α in tumor progression [49]. In this study, in PPAR α (−/−) and wild type mice were compared, showing that the absence of PPAR α in the tumor-infiltrating inflammatory cells lead to the striking inhibition both of primary tumor and of metastasis development in melanoma (B16-BL6, B16-F10) and Lewis Lung Carcinoma mouse models. The tumor growth suppression was mediated by enhanced thrombospondin-1 (TSP-1) production by infiltrating PPAR α deficient immunocompetent cells and subsequent angiogenesis inhibition [49].

Interestingly, the next study by this group demonstrated tumor suppressive action of fenofibrate *in vivo*, which was dependent on PPAR α expression in the tumor stroma, but not on the receptor expression in the tumor itself [50]. Fenofibrate and Wy14,643 inhibited

neovascularization via strong reduction of VEGF and FGF2 secretion with simultaneous enhancement of TSP-1 production [50]. These two studies emphasize the role of PPAR α expression and activity not only in the cancer cells, but also in the host cells present in the tumor bed, such as the infiltrating immune cells and the stromal connective tissue. Taken together, PPAR α ligands can target variety of cells and exert multiple biological actions in the tumor microenvironment. The pleiotropic effects of PPAR α agonists, that can affect both the host and the tumor tissue, include also specific modulations at the level of normal and tumor cell energy metabolism.

4. MOLECULAR BASIS OF CELLULAR RESPONSE TO DIETARY FACTORS: ROLE OF SIRTUINS, AMPK AND PPAR α

The impact of diet on the pattern of metabolic gene expression includes the regulatory mechanisms on both genetic and epigenetic levels. In general, epigenetic alterations in gene expression are those which are not determined by the sequence of genomic DNA, but rather related to the differences in chromatin stability or structure. The main two epigenetic modes of action, that play crucial role in genomic imprinting, embryonic organogenesis and fetal programming, comprise DNA methylation and histone modifications (especially acetylation). Fetal programming refers to mechanisms by which environmental conditions affecting a mother determine the realization of certain genetic program in the offspring. Numerous epidemiological studies describe the correlation of metabolic disorders prevalence like diabetes, cardiovascular disease and obesity with maternal nutrition quality during pregnancy [51]. An experimental animal model developed to study this phenomenon employs a moderate protein restriction in pregnant rats, and was shown to permanently decrease methylation of hepatic PPAR α and glucocorticoid receptor genes in offspring [52, 53]. This hypomethylation resulted in upregulation of genes involved in fatty acid oxidation and gluconeogenesis in young rats and this pattern persisted after weaning until adulthood [54]. The example of fetal programming illustrates how effective dietary treatment can be in modulating the metabolism. Fetal programming in response to mother's dietary choices is important from the evolutionary point of view, as it gives the fetus the chance to adapt to the conditions it would likely come across after birth. The protein restriction or other types of mother undernourishment induce metabolic alterations in offspring that improve survival after birth provided that the environmental conditions are still unfavorable. This is so called 'thrifty phenotype' hypothesis that assumes the metabolic changes to conserve the glucose pool for the brain development and function [55]. However, when after birth the child is exposed to caloric and nutrient excess the existing epigenetically forced gene expression pattern predisposes to type II diabetes mellitus, cardiovascular disease and obesity or all of them in form of the metabolic syndrome, independently of lifestyle risk factors [56, 57].

As excessive nutrient supply is to blame, the first and the simplest recommendation prescribed in such metabolic disorders and obesity is calorie restriction (CR). CR has profound consequences which result in a significant increase of a lifespan observed universally in eukaryotes (yeast, nematodes - *Caenorhabditis elegans*, insects - *Drosophila melanogaster* and vertebrates) [58, 59]. Experiments with rodents demonstrated that besides living longer, CR delayed incidence of various aging-associated diseases including cancer, diabetes, cardiomyopathy, atherosclerosis or autoimmune disorders [60]. Calorie restricted mammals show decreased blood glucose, insulin growth factor 1 (IGF-1) and insulin levels and the inhibition of adipogenesis. On the cellular level, the repression of glycolysis and IGF-1 signaling pathway and activation of gluconeogenesis and mitochondrial biogenesis is observed [61]. Insulin/ IGF-1/ Akt/ mammalian target of rapamycin (mTOR) signaling axis participates in endocrine response to nutritional factors and the turning down these pathways has been shown to prolong life span in *C. elegans* and mice [62, 63]. On the level of cellular energy supply, CR accelerates the rise in NAD^+/NADH and AMP/ATP ratios. The first one

triggers a strong activation of sirtuins, NAD^+ dependent protein deacetylases, and the cecond one activates AMPK [64, 65] Fig. (2).

4.1. The Role of AMPK

AMPK acts as a metabolic check point sensitive to intracellular ATP content, and its main function is to block anabolic processes, such as protein and nucleotide biosynthesis by the activation the tumor suppressor tuberous sclerosis complex 2 (TSC2) - an indirect inhibitor of mTOR [65]. AMPK activation affects also lipid and cholesterol synthesis by phosphorylation - mediated inactivation of the enzymes: acetyl-CoA carboxylase 1 (ACC1) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) [66]. The second aspect of AMPK activity is to mobilize the alternative energy supply in form of fatty acid oxidation and autophagy, with concurrent inhibition of glycolysis by phosphorylation of multiple forms of phosphofructo-2-kinase (PFK2) and by mTOR inhibition [67, 68].

This spectrum of AMPK activity overlaps with the actions mediated by PGC-1α / PPAR α axis, which indicates a functional cooperation among these three proteins. Indeed, the striking convergence in upregulation of fatty acid oxidation has been demonstrated in cell culture experiments with PPAR α and AMPK activators: fibrates and n-3 PUFA increased fatty acid oxidation in AMPK- dependent manner [69, 70], and similarily, 5-aminoimidazole-4-carboxamide-1-β-D-riboside (AICAR), an AMPK activator, stimulated this metabolic pathway through PPAR α and PGC1 α [71].

4.2. The Biological Significance of Sirtuins

Sirtuins are homologs of yeast protein Sir2p (silencing information regulator 2), which were discovered in bacteria and in various species of the animal kingdom, including humans. They act as NAD^+ dependent protein lysine deacetylases, originally known as class III histone deacetylases, as their first identified substrates were histones [72]. SIRT1 is the most studied sirtuin, although in mammals SIRT1 – 7 have been described [59]. SIRTs catalyze the transfer of acetyl group from the protein lysine residue to NAD^+ molecule, which is being broken to 2'- or 3'-O-acetyl-ADP-ribose and nicotinamide. SIRT4 mainly shows ADP-ribosyltransferase instead of deacetylase activity [73]. Nicotinamide and NADH are the inhibitors of sirtuins. The list of SIRT1 substrates reflects the role SIRT1 plays in epigenetic regulation by chromatin modification, and also modulation of gene transcription. It includes histones H1, H3, H4 and numerous transcription factors and proteins involved in signal transduction, like p53, FOXOs, MyoD, PPAR γ, PGC-1α, p300 and nuclear factor kappa B (NFκB) [63].

The sirtuins involvement in the CR - induced lifespan elongation has been best studied in yeast of *SIR2* wild type and with *SIR2* deletion. CR increases both replicative (number of cell divisions before reaching senescence) and chronological lifespan (period of cell viability), but Sir2p takes part only in the first one (reviewed in [59]). Yeast mutants with additional copies of *SIR2* replicate over 30 % longer than wild type yeast, and *SIR2* deletion decreases replicative lifespan by half [74]. Sir2p (−/−) mutants do not show any lifespan increase when subjected to CR. Sir2p mediates epi-genetic silencing by condensing heterochromatin in telomeres, mating type loci and genes coding ribosomal RNA; additionally it contributes to cell cycle control, DNA replication and repair [72, 74, 75].

In mammalian cells SIRT1 is upregulated by CR and shows pleiotropic activities, which in general fall into two main categories: energy sparing and cellular stress managing. The first category includes SIRT1 mediated induction of the tumor suppressor LKB1, the direct upstream regulatory kinase of AMPK; inhibition of PPAR γ dependent adipogenesis; induction of PGC-1α and mitochondrial biogenesis; induction of autophagy and

transcriptional repression of uncoupling protein (UCP2). AMPK, as mentioned above, inhibits glycolysis by blocking IGF-1/Akt/mTOR pathway, and induces fatty acid oxidation in cooperation with PGC-1α and PPAR α [65] Fig. (2).

The second category of stress protecting SIRT1 activities include activation of forkhead box O (FOXO) transcription factors, especially FOXO1, deacetylation of p53, promotion of heterochromatin condensation mediated by histone deacetylation and multidirectional protection of genomic stability by DNA double strand breaks repair through both homologous recombination or nonhomologous end joining (MRE11-RAD50-NBS1, GADD45, Ku70) [59]. In addition, deacetylation of lysine residues in p53 carboxy terminus by SIRT1 impairs p53 DNA binding capability [76].

Regulation of p53 activity by SIRT1 has gained much attention because it may suppress p53 mediated apoptosis and senescence, and was instantly linked to carcinogenesis. However, the role of SIRT1 in transformation and tumor progression is controversial and complex. On one hand, p53 is inactivated, but on the other the inhibition of antiapoptotic PI3K/Akt pathway, as well as activation of transcription factors FOXO takes place. The latter would antagonize growth factor signals by transactivating the genes encoding proapoptotic (BIM), cell cycle inhibitory (p27Kip) and ROS protecting (superoxide dysmutase, MnSOD) proteins [63]. Even though elevated levels of SIRT1 were detected in numerous types of cancer: prostate cancer, non-melanoma skin carcinomas, acute myeloid leukemia and colon cancer, there is a similarly long list of tumors: glioblastoma, bladder carcinoma, ovarian carcinoma and 263 cases of hepatic carcinoma, where SIRT1 level is lower than in respective normal tissues [77, 78]. Therefore, it is still difficult to discriminate if increased SIRT1 expression is a cause or a result of oncogenic transformation. More detailed studies by Lim and coworkers indicate that the SIRT1 contribution to the malignant phenotype is not meaningful, since overexpression of deacetylase silent SIRT1 mutant (H36Y) which leads to abundance of hyperacetylated p53, has no impact on replica-tive life span and DNA damage response [77]. Doubtless, the increased longevity and lower cancer occurrence in animals subjected to CR, the main SIRT1 activator, comparing to those fed *ad libitum*, points to rather beneficial actions of SIRT1.

Additionally, activities of other members of sirtuin family may be attributed to cancer protection. For example, SIRT2 blocked cell cycle during mitotic stress induced by microtubule disrupting agents [79], it has a proapoptotic activity and is downregulated in some cancers [80–82]. SIRT6 activates tumor suppressor gene GCIP and supresses NFκB [83, 84]. Moreover, the mitochondrial sirtuins SIRT3 – 5 may be considered as tumor supressors as they show multiple activities counteracting the cellular transformation.

SIRT3 deacetylases cyclophilin D, a peptidyl-prolyl cistrans isomerase residing in mitochondrial matrix [85]. This isomerase has various biological activities, for example regulates protein folding and the formation of mitochondrial permeability transition pore (MPTP). MPTP possesses three main components: voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane, adenine nucleotide translocator in the inner membrane and cyclophilin D in the mitochondrial matrix in the proximity of the inner membrane. The pore opens in case of cellular stress and causes loss of mitochondrial membrane potential and swelling, which finally causes cell death [86]. In cancer cells VDAC is frequently bound to hexokinase II and this complex is believed to protect from apoptosis, as well as downregulates the mitochondrial oxidative phosphorylation [87]. When cyclophilin D is deacetylated by SIRT3, it relocates from the mitochondrial inner membrane and induces the breakage of VDAC / hexokinase II complex. This event leads to redistribution of hexokinase II from mitochondria to cytoplasm and increased susceptibility of cancer cells to apoptosis [85]. This process is accompanied by the increase in oxidative

phosphorylation intensity [85]. Additionally, SIRT3 plays a role in genotoxic and oxidative stress protection by deacetylation of Ku70 [88]. It also upregulates fatty acid oxidation by deacetylation of long chain acyl-CoA dehydrogenase (LCAD) [89], activates the mitochondrial enzymes: glutamate dehydrogenase, isocitrate dehydrogenase succinate dehydrogenase and acetyl-CoA synthetase 2 [90, 91]. Acetyl-CoA synthetases 1 and 2 (a mitochondrial and a SIRT1 activated cytoplasmic one, respectively) are especially important for the production of acetyl-CoA from excessive amounts of acetate formed during fasting induced ketogenesis [73]. The role of the ketogenesis in cancer cells has a therapeutic significance and will be commented below. On the other side, glutamate dehydrogenase is ADP-ribosylated and inactivated by mitochondrial SIRT4 [92]. Glutamate dehydrogenase is an enzyme of glutaminolytic pathway, which is important for cancer cells, as will be discussed in the section devoted to the cancer cell metabolic characteristics. SIRT5 cooperates with SIRT3 in the enhancement of oxidative metabolism by deacetylation and activation of cytochrome c oxidase [91].

5. METABOLIC CHARACTERISTICS OF TUMOR CELLS

Recently, growing number of attempts has emerged towards understanding of metabolic behavior of cancer cells and to find particular weak points, which in perspective may become a targets for new therapeutic approaches. Metabolic alterations observed within cancer cells result from specific genetic program forced by oncogene expression or are induced by tumor associated microenvironment. The most characteristic feature of tumor cell metabolism is dependency on glucose as the main energetic substrate. It is a consequence of enhanced aerobic glycolysis (known as `Warburg effect') and is frequently accompanied by a high demand and consumption of glutamine (for excellent comprehensive reviews see [93–96].

Glycolysis, despite of being much less efficient in producing energy than oxidative phosphorylation, is far quicker than oxidation of pyruvate in mitochondria. As long as there is unlimited access to glucose, glycolytic tumor cells gain advantage over normal quiescent cells in energy supply. Pyruvate derived from glycolysis is either reduced to lactate or enters the tricarboxylic acid (TCA) cycle, which is a major source of intermediates for fatty acid, cholesterol and isoprenoid biosynthesis (so called cataplerosis) [93]. In cancer cells the balance is shifted towards export of citrate from mitochondria to cytoplasm rather than its oxidation to CO_2, and therefore the pathway is often called “truncated TCA cycle”. Cytoplasmic citrate converted by ATP-citrate lyase (ACL) to acetyl-CoA is a main precursor for fatty acid synthesis. Other TCA intermediates: oxaloacetate and α ketoglutarate are the sources of nonessential aminoacid for protein biosynthesis. This excessive TCA intermediate utilization creates the need for their regeneration termed anaplerosis. Oxaloacetate is generated from pyruvate and nonessential amino acids, such as asparaginate. Alpha ketoglutarate is produced in oxidative deamination of glutamine in the process of glutaminolysis.

Glutaminolysis ends up in converting glutamine to pyruvate, which is then reduced to lactate by lactate dehydrogenase [97]. Intensity of anaplerotic flux is actually a better prognostic of proliferation rate than glycolytic flux, as it indicates the presence of macromolecular synthesis rate and is independent of microenvironmental factors like oxygen concentration [93]. Glutamine is intensely consumed by normal proliferating and tumor cells; the latter uptake the amounts of glutamine that far exceeds the needs for protein and nucleotide biosynthesis. Recently, it has been revealed that the oncogene c-Myc is responsible for enhanced glutamine utilization in glioblastoma cells [97] and restores glutaminase expression by downregulation of repressing miR-23a/b microRNA [98]. In consequence, c-Myc overexpressing cancer cells have their mitochondria programmed to carry on intensive

glutamine catabolism, which can rescue cells from starvation in the state of glucose deprivation [99].

Glucose avidity manifested by an increased glucose uptake and enhanced glycolytic flux result from hyperactivity of oncogenes such as c-Myc, or signaling pathways e.g. IGF-1R/PI3K/Akt/mTOR, and are frequently accompanied by mitochondrial dysfunction [100]. Mitochondrial defects are not only the consequence of hyperactivity of oncogenes but may lead to their activation, as was shown on lymphoma and leukemia cells, which responded to mitochondrial respiration injury by Akt activation [101]. Another circumstance generally regarded as predisposing to enhanced glycolysis is low oxygen concentration inside solid tumors. However, oxygen concentration in the hypoxic regions of human tumors was estimated at 8 – 57 μM, whereas only the concentration lower than 1 μM could significantly affect oxidative phosphorylation efficiency [102]. Indeed, there are relatively few examples of experimental tumor cell lines which rely exclusively on glycolysis, and certain level of oxidative phosphorylation, although lower than in normal tissues, exists in majority of tumors. This means that glycolysis is not merely a mean for generating ATP, but it provides glucose derived intermediates for pentose phosphate pathway which produce ribose-5-phosphate for nucleotide synthesis and NADPH for fatty acid biosynthesis [96, 103].

Avid glucose uptake and consumption by transformed cells is facilitated by abnormally high levels of membrane glucose transporters GLUTs, especially GLUT1 and increased activities of glycolytic enzymes [102]. Oncogenic Akt and its downstream effector mTOR kinase play a central role in programming the glycolytic phenotype by induction of GLUT1 expression and its membrane localization [104], as well as induction of hexokinase I and II association with mitochondria [105]. Akt overexpression is capable of forcing the Warburg effect in both normal and cancer cells [106, 107]. Additionally, Akt is responsible for enhanced fatty acid and cholesterol synthesis by activating the transcription of sterol regulatory element binding protein 1 (SREBP-1) and the lipogenic enzymes: FAS, HMGCR and ACL [108]. Increased lipogenesis in proliferating tumor cells is associated with their high demand for phospholipids as new plasma membrane components and substrates for protein acylation.

Oncogenic c-Myc is a broad functional metabolic regulator which also induces expression of GLUT1, as well as a panel of glycolytic enzymes: hexokinase, PFK, enolase, GAPDH and LDH [109, 110]. In addition to glutaminase upregulation, c-Myc has been reported to regulate glutamine uptake by direct transactivation of glutamine transporters ASCT2 and SN2 [97]. Interestingly, glutaminolysis in c-Myc transformed cells is induced in case of glucose withdrawal or Akt inhibition [99, 111]. Therefore, active glutamine catabolism serves as an energetic rescue pathway which promotes survival in glucose starved cancer cells.

Importantly, metabolic reprogramming of cancer cells results not only from the genetic alterations, but can be induced by tumor microenvironment. A recent study by Sanchez-Arago and colleagues [112] revealed the existence of `bioenergetic signature' in colon carcinoma, which includes downregulation of β-F1 ATPase, increased expression of glycolytic enzymes and repressed mitochondrial respiration. This signature appears in the process of clonal selection at the tumor site in host organism. The decrease of mitochondrial bioenergetic activity in the aggressive clones of colon cancer cells was associated with abnormalities in mitochondrial morphology and resulted from repression of genes involved in mitochondrial biogenesis and function. Such phenotype was required for the tumor progression, accelerated growth, improved cell survival and increased mortality of host animals. The most striking was the observation that in cell culture conditions all the colon carcinoma clones, wild type cells, those with activated oxidative mitochondrial metabolism and those with repressed respiration had the same growth parameters. The conclusion that

might be drawn from these results is that genetic background of transformed cells is not the only factor determining their metabolic preferences, and that the bioenergetic signature of the cancer cell can be modulated by the microenvironment. The influence of the tumor surroundings is at least equally significant, and if so, it opens a possibility for pharmacological treatment targeted at both tumor cells and the stroma.

6. DIETARY AND PHARMACOLOGICAL MODULATION OF TUMOR METABOLISM: A ROLE OF PPARα

The concept of influencing the tumor by inducing changes in its environment lead to the development of various dietary regimens for cancer patients. As this may seem obsolete in comparison to the cutting edge modern drugs designed to hit very specific molecular targets, still this approach may be beneficial for patients. It certainly presents several advantages over the standard chemotherapy: it is much less expensive and practically free of side effects. Moreover, the above mentioned epigenetic and transcriptional mechanisms activated in response to the environmental nutrient availability seem to be able to exert impacts, which are strong enough to effectively supplement canonic anticancer therapies. PPARs with a special emphasis on PPAR α, are good candidates to serve as mitochondria targeted molecular tools for attacking metabolic weak points of the cancer cell.

First, PPAR α is the key inducer of fatty acid oxidation and oxidative metabolism in most tissues, with liver, brain and muscle being the most important ones, where it transactivates numerous genes involved in these pathways, most of which operate in mitochondria: fatty acid binding proteins (FABP), carnitine palmitoyltransferase 1 (CPT1), acyl-CoA oxidase (ACO), acyl-CoA dehydrogenase (ADH), mitochondrial thioesterase 1 (MTE1), just to list few. Next, as reviewed before [113], PPAR α activation contributes to the inhibition of glycolysis by antagonizing IGF-1/Akt signaling [20, 46] and decreasing GLUT1, GLUT2, GLUT4 expression [114–116]. PPAR α blocks specific anabolic pathways: lipogenesis and fatty acid synthesis by a double action. One is the transcriptional repression of FAS, ACC, SREBP-1 and SREBP-2 [117] and the other is through induction of pyruvate dehydrogenase kinase 4 (PDK4) [118] Fig. (3). This kinase is important for the choice of the destination of glycolysis-derived cellular pyruvate pool. Acetyl-CoA molecules generated in oxidative decarboxylation of pyruvate in mitochondria are the building blocks for fatty acid synthesis. This reaction is catalyzed by pyruvate dehydrogenase complex (PDC) and constitutes the rate limiting step for pyruvate entry to mitochondria. Pyruvate dehydrogenase activity is tightly regulated by phosphorylation carried on by pyruvate dehydrogenase kinase isoenzymes PDK1 – PDK4. Phosphorylation makes PDC inactive and this event blocks the glycolysis-derived pyruvate transport to mitochondria and slows down glycolysis. PPAR α synthetic ligands (Wy14,643) and natural PPAR α ligands generated during fasting, increase PDK4 expression and further suppress PDC and acetyl-CoA generation for anaplerosis and fatty acid biosynthesis [118].

Finally, PPAR α decreases mitochondrial aminoacid catabolism by suppressing the transcription of glutaminase and glutamate dehydrogenase in liver [119, 120]. PPAR α activation and fasting state in mice decrease blood urea levels, which reflect the rate of total aminoacid catabolism [119, 121]. These facts indicate that PPAR α activity may counteract c-Myc induced glutaminolysis in cancer cells, and reduce anaplerotic flux from glutamine consumption.

Ultimately PPAR α is activated in fasting and the scope of its molecular targets largely overlaps with SIRT1 and AMPK activities under CR conditions. Actually, PPAR α was shown to act downstream of SIRT1 and mediate SIRT1 – induced promotion of fatty acid oxidation in hepatocytes [5, 122]. The main link between all these players is PGC-1α, which

is downstream element of SIRT1 and AMPK pathways and a partner and coactivator of PPAR α involved in transcriptional regulation Fig. (2).

In attempt to modulate cancer cell metabolism, an interesting dietary approach has been proposed for the treatment of terminal brain tumors that were unsuccessfully managed by conventional therapies [123]. The authors present the idea that the calorie restricted ketogenic (high fat, low carbohydrate) diet (CRKD) in case of malignant glioma and glioblastoma may be far more advantageous than chemotherapy or radiation therapy, which are highly toxic and frequently ineffective. Brain tumors strongly rely on glucose supply from surrounding tissues and usually present a highly glycolytic phenotype. In contrast to normal neurons and glia, which can metabolize ketone bodies (acetoacetate and β-hydroxybutyrate), the tumor cells lack genomic and metabolic flexibility and cannot adapt to the drop in circulating glucose levels. In consequence, they undergo massive apoptosis in the state of energy deprivation. Utilization of ketone bodies as a respiratory substrate is based on functional mitochondria and this requirement is often not supported in glycolytic brain tumors, which therefore would be sensitive to starvation induced by CRKD.

Enhanced oxidative metabolism and ketogenesis are the hallmarks of PPAR α activation induced by fasting [124]. Ketogenesis takes place in mitochondria and refers to the conversion of fatty acid oxidation-derived acetyl-CoA to actoacetate and β-hydroxybutyrate. The enzyme critical for this process is 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS), which transcription is PPAR α dependent [124]. PPAR α agonist ciprofibrate and broader specificity PPAR ligand valproate induce its expression in brain and liver [125]. These facts imply possible efficacy of PPAR α ligands in the treatment of brain tumors.

The CRKD indeed lowers blood glucose and increases ketone bodies levels in patients, and improves the mitochondrial respiratory activity and glutathione redox state in normal cells and therefore may have a neuroprotective effect [126]. Decreased glucose concentrations in brain tissues translate to reduction of glycolysis intensity and lactate production by tumor cells and therefore potentially inhibits inflammatory response at the tumor site, since lactate has been shown to have proinflammatory activity [127, 128].

The experimental verification of CRKD was performed on mice with implanted malignant astrocytoma (CT-27) or human malignant glioma cell line U87-MG, fed on KetoCal, a new diet specially formulated for children with epilepsy [129]. Calorie restricted KetoCal diet significantly decreased the intracerebral growth of both tumor types and decreased the intratumoral microvessel density. Implementation of this diet resulted in elevation of plasma concentrations of ketone bodies, which might trigger the energetic imbalance in the tumors, since tumor tissue showed reduced activity of the enzymes required for ketone body oxidation: β-hydroxybutyrate dehydrogenase and succinyl-CoA: 3-ketoacid-CoA transferase comparing to conlateral normal brain tissue [129]. Moreover, in some cases of advanced malignant tumors (anaplastic astrocytoma and cerebellar astrocytoma), patients respond well to CRKD dietary regimen [123]. The question about the safety of CRKD in patients who are likely to develop cachexia due to tumor burden may arise, nevertheless malnutrition or undernourishment have not been reported [123].

Remarkably, ketogenic diet is also beneficial for patients with neurological disorders, especially in epilepsia [130]. The first observations revealed that starvation – mimicking diet, and CRKD in particular, have anti seizure properties [131, 132]. Further investigation stated that both high fat content and reduced total caloric intake are important because they induce hormonal responses favoring ketogenesis: low insulin and high glucagon, as well as increased cortisol blood levels promote acetyl-CoA conversion to ketone bodies and release to circulation [131]. Increase in blood fatty acid concentration, which physiologically

activates PPAR α, was observed in CRKD as a result of fat reserves mobilization and high fat intake [131] Fig. (3). Ketone bodies are avidly consumed by brain tissue during glucose deprivation. In limited glucose availability, astrocytes protect neurons from the energetic stress by performing fatty acid oxidation and ketogenesis and supply the surrounding neurons with ketone bodies [133–136]. Ketone bodies are prioritized energetic substrates and they are metabolized before glucose and free fatty acids. Their cellular uptake is mediated by monocarboxylate transporter MCT1, which transcription is positively regulated by PPAR α [137]. Importantly, both endogenous (free fatty acids) and synthetic PPAR α ligands are free to flow through the blood-brain barrier and they may reach high levels in the brain tissue [136].

In addition to the role in brain tumors, ketogenesis may also become a prognostic factor in colon carcinoma. 3-Hydroxy-3-methylglutaryl-CoA synthase is severely down-regulated by c-Myc in colon cancer cell lines with high activity of Wnt/ β-catenin/ T cell factor 4 (TCF-4)/ c-Myc pathway [138]. Ketogenic capability and HMGCS expression levels are positively correlated with enterocyte differentiation and decreased in colon or rectal carcinomas, especially those poorly differentiated [138].

In theory, PPAR α activation could counteract c-Myc induced alterations of mitochondrial metabolism by restoring the HMGCS and ketogenesis levels and by inhibiting glutaminolysis through transcriptional repression of the two enzymes crucial for this pathway: glutaminase and glutamate dehydrogenase. Moreover, PPAR α and pan-PPAR agonists like bezafibrate stimulate oxidative phosphorylation and respiratory capacity by inducing PGC-1α mediated mitochondrial biogenesis [139]. Although this activity goes in line with c-Myc action, which was reported to stimulate mitochondrial proliferation, this could cause either normalization of cancer cell energetic balance or induce a `metabolic catastrophe' in the cells with genetically impaired mitochondrial function.

Recent studies on mice bearing different tumors revealed that dietary restrictions do not affect those with constitutive activation of PI3K/Akt pathway [140]. Other cancer characterized by transformed *HRAS*/ *KRAS, BRAF* or with loss of *TP53*, show a significant decrease of the growth rate and increased apoptosis when the host animals were subjected to dietary restriction. Resistance to dietary restriction depended on the Akt phosphorylation status and its activation, which lead to FOXO1 phoshorylation and cytoplasmic sequestration [140]. When arrested in the cytoplasm, FOXO1 is unable to exert its proapoptotic functions [141].

There are several proteins that negatively affect Akt activity, namely protein phosphatases PTEN, SHIP and PPA2 that directly dephoshorylate Akt; and TRB3 (mammalian homolog of *Drosophila protein tribbles*), a protein that binds to Akt and blocks its phosphorylation [142–147]. TRB3 expression in liver rises during fasting and is driven by PGC-1α in PPAR α dependent manner, as there are functional PPRE elements in the TRB3 promoter [148]. Metabolic function of TRB3 is to block insulin dependent Akt activation in fasting, in parallel to PGC-1α / PPAR α induced gluconeogenesis and fatty acid oxidation. This seems to be a part of a SIRT1/ LKB1/ AMPK/ PGC-1α pathway which constitutes an adaptive response to CR, because in mice with muscle – specific *LKB1* knockout in which the PGC-1α, PPAR α and TRB3 were severely decreased [149]. Interestingly, TRB3 upregulation in lymphocytes is induced by fibrates in PPAR α independent fashion with utilization of C/EBP and C/EBP homologous proteins [150].

Therefore it is reasonable to speculate that pharmacological PPAR α activation together with CRKD might improve the anticancer outcome in case of dietary restriction resistant tumors with overactive PI3K or nonfunctional PTEN. The situation would probably be

different in tumors with constitutively active plasma membrane associated Akt mutants (with activating Akt mutations or in model systems with the introduction of myristoylated Akt), as in these cases TRB3 possibly would not affect the already phosphorylated Akt. Although these hypotheses need to be verified, the negative influence of TRB3 on Akt phosphorylation seems to be a general phenomenon.

7. CONCLUSION

PPAR α activators have a great potential of supplementing conventional anticancer therapies by modulating cancer cell energy metabolism and signaling pathways. This notion is based on multiple observations, which include PPAR α-mediated inhibition of two prominent oncogenes: c-Myc and Akt Fig. (3). In this inhibitory action, PPAR α suppresses glutaminolysis and glutamine catabolism in mitochondria, as well as activates ketogenesis by promotion of fatty acid oxidation and transactivation of HMGCS. In cancer cells these processes are c-Myc regulated. Next, PPAR α actions slow down glycolysis by inhibiting Akt and blocks Akt induced fatty acid synthesis by repressing PDH activity in mitochondria via PDK4 upregulation. Finally PPAR α functionally cooperates with AMPK, SIRT1 and PGC-1α in regulating the cellular response to calorie restriction. In perspective, it would be important to elucidate the details of possible interplay between these regulatory proteins, and to verify the role of PPAR α activation in the of CRKD applied to *in vivo* cancer models.

Of note, the potential use of clinically tested synthetic ligands for PPAR α against cancer, although seems to be a straightforward and fairly safe procedure, it still requires our careful consideration. There are still debates over fibrate drugs safety and three main caveats have been are presented [151]: (1) fibrate ability to bind hemoglobin which reduces its affinity to oxygen; (2) fibrates may disrupt mitochondrial electron transfer chain particularly by inhibiting complex I; and (3) fibrates induce mitochondrial ROS production. These potentially harmful activities are presently understood to be receptor-independent, which implies the need for new generation of PPAR α ligands which would possess improved physiological and pharmacological characteristics.

Acknowledgments

The authors are supported by the Polish Ministry of Science and Higher Education (grant no. N N302 130834 to M. P.) and the US National Institutes of Health (grant no. RO1 CA 095518 to K. R.)

LIST OF ABBREVIATIONS

ACC	Acyl-CoA carboxylase
ACL	ATP-citrate lyase
ACO	Acyl-CoA oxidase
ADH	Acyl-CoA dehydrogenase
AMPK	AMP-dependent protein kinase
CPT1	carnitine palmitoyltransferase 1
CR	Calorie restriction
CRKD	Calorie restricted ketogenic diet
FABP	Fatty acid binding protein
FAS	Fatty acid synthase

FOXO	Forkhead box class O
GABA	Gamma butyric acid
GAPDH	Gliceraldehyde dehydrogenase
HDAC	Histone deacetylase
HDL	High density lipoprotein
HMGCR	3-Hydroxy-3-methylglutaryl-CoA reductase
HMGCS	3-Hydroxy-3-methylglutaryl-CoA synthase
IGF-1	Insulin growth factor 1
LCAD	Long chain acyl-CoA dehydrogenase
LDH	Lactate dehydrogenase
LDL	Low density lipoptotein
MCT1	Monocarboxylate transporter 1
MnSOD	Manganese superoxide dysmutase
MTE1	Mitochondrial thioesterase1
mTOR	Mammalian target of rapamycin
PDC	Pyruvate dehydrogenase complex
PDH	Pyruvate dehydrogenase
PDK	Pyruvate dehydrogenase kinase
PEPCK	Phosphoenol pyruvate carboxykinase
PFK	Phosphofructo kinase
PGC-1	Peroxisome proliferator activated receptor γ coactivator 1
PI3K	Phosphoinositol-3 kinase
PPAR	peroxisome proliferator activated receptor
PTEN	Phosphatase and tensin homolog
ROS	Reactive oxygen species
RXR	Retinoid × receptor
SIRT1	Sirtuin 1
SREBP-1	Sterol regulatory element binding protein 1
TCA	Tricarboxylic acid cycle
TCF-4	T cell factor 4
TSC2	Tuberous sclerosis complex 2
UCP2	Uncoupling protein 2

REFERENCES

[1]. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev. 1999; 20:649–688. [PubMed: 10529898]

[2]. Escher P, Wahli W. Peroxisome proliferator-activated receptors: insight into multiple cellular functions. Mutat Res. 2000; 448:121–138. [PubMed: 10725467]

[3]. Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev. 2000; 14:121–141. [PubMed: 10652267]

[4]. Blanquart C, Mansouri R, Paumelle R, Fruchart JC, Staels B, Glineur C. The protein kinase C signaling pathway regulates a molecular switch between transactivation and transrepression activity of the peroxisome proliferator-activated receptor alpha. Mol Endocrinol. 2004; 18:1906–1918. [PubMed: 15131257]

[5]. Sugden MC, Caton PW, Holness MJ. PPAR control: it's SIRTainly as easy as PGC. J Endocrinol. 2010; 204:93–104. [PubMed: 19770177]

[6]. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature. 2005; 437:759–763. [PubMed: 16127449]

[7]. Ghisletti S, Huang W, Ogawa S, Pascual G, Lin ME, Will-son TM, Rosenfeld MG, Glass CK. Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. Mol Cell. 2007; 25:57–70. [PubMed: 17218271]

[8]. Frederiksen KS, Wulff EM, Sauerberg P, Mogensen JP, Jeppesen L, Fleckner J. Prediction of PPAR-alpha ligand-mediated physiological changes using gene expression profiles. J Lipid Res. 2004; 45:592–601. [PubMed: 14999041]

[9]. Ribet C, Montastier E, Valle C, Bezaire V, Mazzucotelli A, Mairal A, Viguerie N, Langin D. Peroxisome proliferator-activated receptor-alpha control of lipid and glucose metabolism in human white adipocytes. Endocrinology. 151:123–133. [PubMed: 19887568]

[10]. Muoio DM, Way JM, Tanner CJ, Winegar DA, Kliewer SA, Houmard JA, Kraus WE, Dohm GL. Peroxisome proliferator-activated receptor-alpha regulates fatty acid utilization in primary human skeletal muscle cells. Diabetes. 2002; 51:901–909. [PubMed: 11916905]

[11]. Luquet S, Lopez-Soriano J, Holst D, Fredenrich A, Melki J, Rassoulzadegan M, Grimaldi PA. Peroxisome proliferator-activated receptor delta controls muscle development and oxidative capability. Faseb J. 2003; 17:2299–2301. [PubMed: 14525942]

[12]. Remels AH, Langen RC, Schrauwen P, Schaart G, Schols AM, Gosker HR. Regulation of mitochondrial biogenesis during myogenesis. Mol Cell Endocrinol. 2010; 315:113–120. [PubMed: 19804813]

[13]. Wang P, Liu J, Li Y, Wu S, Luo J, Yang H, Subbiah R, Chatham J, Zhelyabovska O, Yang Q. Peroxisome Proliferator-Activated Receptor {delta} Is an Essential Transcriptional Regulator for Mitochondrial Protection and Biogenesis in Adult Heart. Circ Res. 2010; 106:911–919. [PubMed: 20075336]

[14]. Keelan JA, Sato TA, Marvin KW, Lander J, Gilmour RS, Mitchell MD. 15-Deoxy-Delta(12,14)-prostaglandin J(2), a ligand for peroxisome proliferator-activated receptor-gamma, induces apoptosis in JEG3 choriocarcinoma cells. Biochem Biophys Res Commun. 1999; 262:579–585. [PubMed: 10471366]

[15]. Marx N, Sukhova GK, Collins T, Libby P, Plutzky J. PPARalpha activators inhibit cytokine-induced vascular cell adhesion molecule-1 expression in human endothelial cells. Circulation. 1999; 99:3125–3131. [PubMed: 10377075]

[16]. Mossner R, Schulz U, Kruger U, Middel P, Schinner S, Fuzesi L, Neumann C, Reich K. Agonists of peroxisome proliferator-activated receptor gamma inhibit cell growth in malignant melanoma. J Invest Dermatol. 2002; 119:576–582. [PubMed: 12230498]

[17]. Placha W, Gil D, Dembinska-Kiec A, Laidler P. The effect of PPARgamma ligands on the proliferation and apoptosis of human melanoma cells. Melanoma Res. 2003; 13:447–456. [PubMed: 14512786]

[18]. Grabacka M, Placha W, Plonka PM, Pajak S, Urbanska K, Laidler P, Slominski A. Inhibition of melanoma metastases by fenofibrate. Arch Dermatol Res. 2004; 296:54–58. [PubMed: 15278363]

[19]. Grabacka M, Placha W, Urbanska K, Laidler P, Plonka PM, Reiss K. PPAR gamma regulates MITF and beta-catenin expression and promotes a differentiated phenotype in mouse melanoma S91. Pigment Cell Melanoma Res. 2008; 21:388–396. [PubMed: 18444964]

[20]. Grabacka M, Plonka PM, Urbanska K, Reiss K. Peroxisome proliferator-activated receptor alpha activation decreases metastatic potential of melanoma cells *in vitro* via down-regulation of Akt. Clin Cancer Res. 2006; 12:3028–3036. [PubMed: 16707598]

[21]. Drukala J, Urbanska K, Wilk A, Grabacka M, Wybieralska E, Del Valle L, Madeja Z, Reiss K. ROS accumulation and IGF-IR inhibition contribute to fenofibrate/PPARalpha -mediated inhibition of Glioma cell notility *in vitro*. Mol Cancer. 2010; 9:159. [PubMed: 20569465]

[22]. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Len-hard JM, Lehmann JM. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. Proc Natl Acad Sci U S A. 1997; 94:4318–4323. [PubMed: 9113987]

[23]. Yu Z, Schneider C, Boeglin WE, Brash AR. Epidermal lipoxygenase products of the hepoxilin pathway selectively activate the nuclear receptor PPARalpha. Lipids. 2007; 42:491–497. [PubMed: 17436029]

[24]. Ng VY, Huang Y, Reddy LM, Falck JR, Lin ET, Kroetz DL. Cytochrome P450 eicosanoids are activators of peroxisome proliferator-activated receptor alpha. Drug Metab Dispos. 2007; 35:1126–1134. [PubMed: 17431031]

[25]. Muga SJ, Thuillier P, Pavone A, Rundhaug JE, Boeglin WE, Jisaka M, Brash AR, Fischer SM. 8S-lipoxygenase products activate peroxisome proliferator-activated receptor alpha and induce differentiation in murine keratinocytes. Cell Growth Differ. 2000; 11:447–454. [PubMed: 10965849]

[26]. Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc Natl Acad Sci U S A. 1997; 94:4312–4317. [PubMed: 9113986]

[27]. Narala VR, Adapala RK, Suresh MV, Brock TG, Peters-Golden M, Reddy RC. Leukotriene B4 is a physiologically relevant endogenous peroxisome proliferator-activated receptor-{alpha} agonist. J Biol Chem. 2010

[28]. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W. The PPARalpha-leukotriene B4 pathway to inflammation control. Nature. 1996; 384:39–43. [PubMed: 8900274]

[29]. Yuri T, Danbara N, Tsujita-Kyutoku M, Fukunaga K, Takada H, Inoue Y, Hada T, Tsubura A. Dietary docosahexaenoic acid suppresses N-methyl-N-nitrosourea-induced mammary carcinogenesis in rats more effectively than eicosapentaenoic acid. Nutr Cancer. 2003; 45:211–217. [PubMed: 12881016]

[30]. Toriyama-Baba H, Iigo M, Asamoto M, Iwahori Y, Park CB, Han BS, Takasuka N, Kakizoe T, Ishikawa C, Yazawa K, Araki E, Tsuda H. Organotropic chemopreventive effects of n-3 unsaturated fatty acids in a rat multi-organ carcinogenesis model. Jpn J Cancer Res. 2001; 92:1175–1183. [PubMed: 11714441]

[31]. Noguchi M, Minami M, Yagasaki R, Kinoshita K, Earashi M, Kitagawa H, Taniya T, Miyazaki I. Chemoprevention of DMBA-induced mammary carcinogenesis in rats by low-dose EPA and DHA. Br J Cancer. 1997; 75:348–353. [PubMed: 9020478]

[32]. Latham P, Lund EK, Johnson IT. Dietary n-3 PUFA increases the apoptotic response to 1,2-dimethylhydrazine, reduces mitosis and suppresses the induction of carcinogenesis in the rat colon. Carcinogenesis. 1999; 20:645–650. [PubMed: 10223194]

[33]. Shahidi F, Miraliakbari H. Omega-3 (n-3) fatty acids in health and disease: Part 1--cardiovascular disease and cancer. J Med Food. 2004; 7:387–401. [PubMed: 15671680]

[34]. Peters JM, Cheung C, Gonzalez FJ. Peroxisome proliferator-activated receptor-alpha and liver cancer: where do we stand? J Mol Med. 2005; 83:774–785. [PubMed: 15976920]

[35]. Shah YM, Morimura K, Yang Q, Tanabe T, Takagi M, Gonzalez FJ. Peroxisome proliferator-activated receptor alpha regulates a microRNA-mediated signaling cascade responsible for hepatocellular proliferation. Mol Cell Biol. 2007; 27:4238–4247. [PubMed: 17438130]

[36]. Gardette V, Bongard V, Dallongeville J, Arveiler D, Bing-ham A, Ruidavets JB, Amouyel P, Haas B, Ducimetiere P, Ferrieres J. Ten-year all-cause mortality in presumably healthy subjects on lipid-lowering drugs (from the Prospective Epidemiological Study of Myocardial Infarction [PRIME] prospective cohort). Am J Cardiol. 2009; 103:381–386. [PubMed: 19166693]

[37]. Demierre MF. What about chemoprevention for melanoma? Curr Opin Oncol. 2006; 18:180–184. [PubMed: 16462188]

[38]. Dellavalle RP, Nicholas MK, Schilling LM. Melanoma chemoprevention: a role for statins or fibrates? Am J Ther. 2003; 10:203–210. [PubMed: 12756427]

[39]. Freeman SR, Drake AL, Heilig LF, Graber M, McNealy K, Schilling LM, Dellavalle RP. Statins, fibrates, and melanoma risk: a systematic review and meta-analysis. J Natl Cancer Inst. 2006; 98:1538–1546. [PubMed: 17077356]

[40]. Shigeto T, Yokoyama Y, Xin B, Mizunuma H. Peroxisome proliferator-activated receptor alpha and gamma ligands inhibit the growth of human ovarian cancer. Oncol Rep. 2007; 18:833–840. [PubMed: 17786343]

[41]. Yokoyama Y, Xin B, Shigeto T, Umemoto M, Kasai-Sakamoto A, Futagami M, Tsuchida S, Al-Mulla F, Mizunuma H. Clofibric acid, a peroxisome proliferator-activated receptor alpha ligand, inhibits growth of human ovarian cancer. Mol Cancer Ther. 2007; 6:1379–1386. [PubMed: 17431116]

[42]. Tuller ER, Brock AL, Yu H, Lou JR, Benbrook DM, Ding WQ. PPARalpha signaling mediates the synergistic cytotoxicity of clioquinol and docosahexaenoic acid in human cancer cells. Biochem Pharmacol. 2009; 77:1480–1486. [PubMed: 19426685]

[43]. Saidi SA, Holland CM, Charnock-Jones DS, Smith SK. *In vitro* and *in vivo* effects of the PPAR-alpha agonists fenofibrate and retinoic acid in endometrial cancer. Mol Cancer. 2006; 5:13. [PubMed: 16569247]

[44]. Zak Z, Gelebart P, Lai R. Fenofibrate induces effective apoptosis in mantle cell lymphoma by inhibiting the TNFalpha/NF-kappaB signaling axis. Leukemia.

[45]. Strakova N, Ehrmann J, Bartos J, Malikova J, Dolezel J, Kolar Z. Peroxisome proliferator-activated receptors (PPAR) agonists affect cell viability, apoptosis and expression of cell cycle related proteins in cell lines of glial brain tumors. Neoplasma. 2005; 52:126–136. [PubMed: 15800711]

[46]. Urbanska K, Pannizzo P, Grabacka M, Croul S, Del Valle L, Khalili K, Reiss K. Activation of PPARalpha inhibits IGF-I-mediated growth and survival responses in medulloblastoma cell lines. Int J Cancer. 2008; 123:1015–1024. [PubMed: 18546270]

[47]. Wu J, Song Y, Li H, Chen J. Rhabdomyolysis associated with fibrate therapy: review of 76 published cases and a new case report. Eur J Clin Pharmacol. 2009; 65:1169–1174. [PubMed: 19756555]

[48]. Maiguma T, Fujisaki K, Itoh Y, Makino K, Teshima D, Takahashi-Yanaga F, Sasaguri T, Oishi R. Cell-specific toxicity of fibrates in human embryonal rhabdomyosarcoma cells. Naunyn Schmiedebergs Arch Pharmacol. 2003; 367:289–296. [PubMed: 12644902]

[49]. Kaipainen A, Kieran MW, Huang S, Butterfield C, Bielen-berg D, Mostoslavsky G, Mulligan R, Folkman J, Panigrahy D. PPARalpha deficiency in inflammatory cells suppresses tumor growth. PLoS ONE. 2007; 2:e260. [PubMed: 17327920]

[50]. Panigrahy D, Kaipainen A, Huang S, Butterfield CE, Barnes CM, Fannon M, Laforme AM, Chaponis DM, Folkman J, Kieran MW. PPARalpha agonist fenofibrate suppresses tumor growth through direct and indirect angiogenesis inhibition. Proc Natl Acad Sci U S A. 2008; 105:985–990. [PubMed: 18199835]

[51]. Chmurzynska A. Fetal programming: link between early nutrition, DNA methylation, and complex diseases. Nutr Rev. 68:87–98. [PubMed: 20137054]

[52]. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. J Nutr. 2005; 135:1382–1386. [PubMed: 15930441]

[53]. Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. Br J Nutr. 2007; 97:1064–1073. [PubMed: 17433129]

[54]. Holness MJ, Caton PW, Sugden MC. Acute and long-term nutrient-led modifications of gene expression: Potential role of SIRT1 as a central co-ordinator of short and longer-term programming of tissue function. Nutrition. 2010

[55]. Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull. 2001; 60:5–20. [PubMed: 11809615]

[56]. Drake AJ, Walker BR. The intergenerational effects of fetal programming: non-genomic mechanisms for the inheritance of low birth weight and cardiovascular risk. J Endocrinol. 2004; 180:1–16. [PubMed: 14709139]

[57]. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev. 2005; 85:571–633. [PubMed: 15788706]

[58]. Kennedy BK, Steffen KK, Kaeberlein M. Ruminations on dietary restriction and aging. Cell Mol Life Sci. 2007; 64:1323–1328. [PubMed: 17396225]

[59]. Vaquero A, Reinberg D. Calorie restriction and the exercise of chromatin. Genes Dev. 2009; 23:1849–1869. [PubMed: 19608767]

[60]. Hursting SD, Lavigne JA, Berrigan D, Perkins SN, Bar-rett JC. Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans. Annu Rev Med. 2003; 54:131–152. [PubMed: 12525670]

[61]. Moore T, Beltran L, Carbajal S, Strom S, Traag J, Hursting SD, DiGiovanni J. Dietary energy balance modulates signaling through the Akt/mammalian target of rapamycin pathways in multiple epithelial tissues. Cancer Prev Res (Phila Pa). 2008; 1:65–76.

[62]. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A C. elegans mutant that lives twice as long as wild type. Nature. 1993; 366:461–464. [PubMed: 8247153]

[63]. Yang T, Fu M, Pestell R, Sauve AA. SIRT1 and endocrine signaling. Trends Endocrinol Metab. 2006; 17:186–191. [PubMed: 16684606]

[64]. Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature. 2009; 458:1056–1060. [PubMed: 19262508]

[65]. Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. Nat Rev Cancer. 2009; 9:563–575. [PubMed: 19629071]

[66]. Carling D, Zammit VA, Hardie DG. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. FEBS Lett. 1987; 223:217–222. [PubMed: 2889619]

[67]. Marsin AS, Bertrand L, Rider MH, Deprez J, Beauloye C, Vincent MF, Van den Berghe G, Carling D, Hue L. Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. Curr Biol. 2000; 10:1247–1255. [PubMed: 11069105]

[68]. Almeida A, Moncada S, Bolanos JP. Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. Nat Cell Biol. 2004; 6:45–51. [PubMed: 14688792]

[69]. Motawi TM, Hashem RM, Rashed LA, El-Razek SM. Comparative study between the effect of the peroxisome proliferator activated receptor-alpha ligands fenofibrate and n-3 polyunsatu-rated fatty acids on activation of 5'-AMP-activated protein kinase-alpha1 in high-fat fed rats. J Pharm Pharmacol. 2009; 61:1339–1346. [PubMed: 19814866]

[70]. Suchankova G, Tekle M, Saha AK, Ruderman NB, Clarke SD, Gettys TW. Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. Biochem Biophys Res Commun. 2005; 326:851–858. [PubMed: 15607747]

[71]. Lee WJ, Kim M, Park HS, Kim HS, Jeon MJ, Oh KS, Koh EH, Won JC, Kim MS, Oh GT, Yoon M, Lee KU, Park JY. AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARalpha and PGC-1. Biochem Biophys Res Commun. 2006; 340:291–295. [PubMed: 16364253]

[72]. Blander G, Guarente L. The Sir2 family of protein deacetylases. Annu Rev Biochem. 2004; 73:417–435. [PubMed: 15189148]

[73]. Yu J, Auwerx J. The role of sirtuins in the control of metabolic homeostasis. Ann N Y Acad Sci. 2009; 1173(Suppl 1):E10–19. [PubMed: 19751409]

[74]. Steinkraus KA, Kaeberlein M, Kennedy BK. Replicative aging in yeast: the means to the end. Annu Rev Cell Dev Biol. 2008; 24:29–54. [PubMed: 18616424]

[75]. Guarente L. Sir2 links chromatin silencing, metabolism, and aging. Genes Dev. 2000; 14:1021–1026. [PubMed: 10809662]

[76]. Luo J, Li M, Tang Y, Laszkowska M, Roeder RG, Gu W. Acetylation of p53 augments its site-specific DNA binding both *in vitro* and *in vivo*. Proc Natl Acad Sci U S A. 2004; 101:2259–2264. [PubMed: 14982997]

[77]. Lim CS. SIRT1: tumor promoter or tumor suppressor? Med Hypotheses. 2006; 67:341–344. [PubMed: 16546327]

[78]. Deng CX. SIRT1, is it a tumor promoter or tumor suppressor? Int J Biol Sci. 2009; 5:147–152. [PubMed: 19173036]

[79]. Inoue T, Hiratsuka M, Osaki M, Yamada H, Kishimoto I, Yamaguchi S, Nakano S, Katoh M, Ito H, Oshimura M. SIRT2, a tubulin deacetylase, acts to block the entry to chromo-some condensation in response to mitotic stress. Oncogene. 2007; 26:945–957. [PubMed: 16909107]

[80]. Hiratsuka M, Inoue T, Toda T, Kimura N, Shirayoshi Y, Kamitani H, Watanabe T, Ohama E, Tahimic CG, Kurimasa A, Oshimura M. Proteomics-based identification of differentially expressed genes in human gliomas: down-regulation of SIRT2 gene. Biochem Biophys Res Commun. 2003; 309:558–566. [PubMed: 12963026]

[81]. Allison SJ, Milner J. SIRT3 is pro-apoptotic and participates in distinct basal apoptotic pathways. Cell Cycle. 2007; 6:2669–2677. [PubMed: 17957139]

[82]. Lennerz V, Fatho M, Gentilini C, Frye RA, Lifke A, Ferel D, Wolfel C, Huber C, Wolfel T. The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. Proc Natl Acad Sci U S A. 2005; 102:16013–16018. [PubMed: 16247014]

[83]. Ma W, Stafford LJ, Li D, Luo J, Li X, Ning G, Liu M. GCIP/CCNDBP1, a helix-loop-helix protein, suppresses tumori-genesis. J Cell Biochem. 2007; 100:1376–1386. [PubMed: 17131381]

[84]. Kawahara TL, Michishita E, Adler AS, Damian M, Berber E, Lin M, McCord RA, Ongaigui KC, Boxer LD, Chang HY, Chua KF. SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. Cell. 2009; 136:62–74. [PubMed: 19135889]

[85]. Shulga N, Wilson-Smith R, Pastorino JG. Sirtuin-3 deacetylation of cyclophilin D induces dissociation of hexokinase II from the mitochondria. J Cell Sci. 2010; 123:894–902. [PubMed: 20159966]

[86]. Tsujimoto Y, Shimizu S. Role of the mitochondrial membrane permeability transition in cell death. Apoptosis. 2007; 12:835–840. [PubMed: 17136322]

[87]. Pedersen PL. Voltage dependent anion channels (VDACs): a brief introduction with a focus on the outer mitochondrial compartment's roles together with hexokinase-2 in the “Warburg effect” in cancer. J Bioenerg Biomembr. 2008; 40:123–126. [PubMed: 18780167]

[88]. Sundaresan NR, Samant SA, Pillai VB, Rajamohan SB, Gupta MP. SIRT3 is a stress-responsive deacetylase in cardio-myocytes that protects cells from stress-mediated cell death by deacetylation of Ku70. Mol Cell Biol. 2008; 28:6384–6401. [PubMed: 18710944]

[89]. Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, Grueter CA, Harris C, Biddinger S, Ilkayeva OR, Stevens RD, Li Y, Saha AK, Ruderman NB, Bain JR, Newgard CB, Farese RV Jr. Alt FW, Kahn CR, Verdin E. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature. 2010; 464:121–125. [PubMed: 20203611]

[90]. Hallows WC, Lee S, Denu JM. Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. Proc Natl Acad Sci U S A. 2006; 103:10230–10235. [PubMed: 16790548]

[91]. Schlicker C, Gertz M, Papatheodorou P, Kachholz B, Becker CF, Steegborn C. Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5. J Mol Biol. 2008; 382:790–801. [PubMed: 18680753]

[92]. Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christo-doulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Wein-druch R, Alt FW, Guarente L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell. 2006; 126:941–954. [PubMed: 16959573]

[93]. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. 2008; 7:11–20. [PubMed: 18177721]

[94]. Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell. 2008; 13:472–482. [PubMed: 18538731]

[95]. Young CD, Anderson SM. Sugar and fat - that's where it's at: metabolic changes in tumors. Breast Cancer Res. 2008; 10:202. [PubMed: 18304378]

[96]. Weinberg F, Chandel NS. Mitochondrial metabolism and cancer. Ann N Y Acad Sci. 2009; 1177:66–73. [PubMed: 19845608]

[97]. Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, Thompson CB. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci U S A. 2008; 105:18782–18787. [PubMed: 19033189]

[98]. Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, Dang CV. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature. 2009; 458:762–765. [PubMed: 19219026]

[99]. Yang C, Sudderth J, Dang T, Bachoo RM, McDonald JG, DeBerardinis RJ. Glioblastoma cells require glutamate dehydrogenase to survive impairments of glucose metabolism or Akt signaling. Cancer Res. 2009; 69:7986–7993. [PubMed: 19826036]

[100]. Ortega AD, Sanchez-Arago M, Giner-Sanchez D, Sanchez-Cenizo L, Willers I, Cuezva JM. Glucose avidity of carcinomas. Cancer Lett. 2009; 276:125–135. [PubMed: 18790562]

[101]. Pelicano H, Xu RH, Du M, Feng L, Sasaki R, Carew JS, Hu Y, Ramdas L, Hu L, Keating MJ, Zhang W, Plunkett W, Huang P. Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. J Cell Biol. 2006; 175:913–923. [PubMed: 17158952]

[102]. Moreno-Sanchez R, Rodriguez-Enriquez S, Marin-Hernandez A, Saavedra E. Energy metabolism in tumor cells. Febs J. 2007; 274:1393–1418. [PubMed: 17302740]

[103]. Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB. Brick by brick: metabolism and tumor cell growth. Curr Opin Genet Dev. 2008; 18:54–61. [PubMed: 18387799]

[104]. Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB. Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. Mol Cell Biol. 2003; 23:7315–7328. [PubMed: 14517300]

[105]. Robey RB, Hay N. Mitochondrial hexokinases: guardians of the mitochondria. Cell Cycle. 2005; 4:654–658. [PubMed: 15846094]

[106]. Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, Zhuang H, Cinalli RM, Alavi A, Rudin CM, Thompson CB. Akt stimulates aerobic glycolysis in cancer cells. Cancer Res. 2004; 64:3892–3899. [PubMed: 15172999]

[107]. Plas DR, Thompson CB. Akt-dependent transformation: there is more to growth than just surviving. Oncogene. 2005; 24:7435–7442. [PubMed: 16288290]

[108]. Porstmann T, Griffiths B, Chung YL, Delpuech O, Griffiths JR, Downward J, Schulze A. PKB/Akt induces transcription of enzymes involved in cholesterol and fatty acid biosynthesis via activation of SREBP. Oncogene. 2005; 24:6465–6481. [PubMed: 16007182]

[109]. Shim H, Dolde C, Lewis BC, Wu CS, Dang G, Jungmann RA, Dalla-Favera R, Dang CV. c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. Proc Natl Acad Sci U S A. 1997; 94:6658–6663. [PubMed: 9192621]

[110]. Kim JW, Zeller KI, Wang Y, Jegga AG, Aronow BJ, O'Donnell KA, Dang CV. Evaluation of myc E-box phylogenetic footprints in glycolytic genes by chromatin immunoprecipitation assays. Mol Cell Biol. 2004; 24:5923–5936. [PubMed: 15199147]

[111]. Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. Clin Cancer Res. 2009; 15:6479–6483. [PubMed: 19861459]

[112]. Sanchez-Arago M, Chamorro M, Cuezva JM. Selection of cancer cells with repressed mitochondria triggers colon cancer progression. Carcinogenesis. 2010

[113]. Grabacka M, Reiss K. Anticancer Properties of PPARalpha-Effects on Cellular Metabolism and Inflammation. PPAR Res. 2008; 2008:930705. [PubMed: 18509489]

[114]. Finck BN, Kelly DP. Peroxisome proliferator-activated receptor alpha (PPARalpha) signaling in the gene regulatory control of energy metabolism in the normal and diseased heart. J Mol Cell Cardiol. 2002; 34:1249–1257. [PubMed: 12425323]

[115]. Oosterveer MH, Grefhorst A, van Dijk TH, Havinga R, Staels B, Kuipers F, Groen AK, Reijngoud DJ. Fenofibrate simultaneously induces hepatic fatty acid oxidation, synthesis, and elongation in mice. J Biol Chem. 2009; 284:34036–34044. [PubMed: 19801551]

[116]. Ahmed W, Ziouzenkova O, Brown J, Devchand P, Francis S, Kadakia M, Kanda T, Orasanu G, Sharlach M, Zandbergen F, Plutzky J. PPARs and their metabolic modulation: new mechanisms for transcriptional regulation? J Intern Med. 2007; 262:184–198. [PubMed: 17645586]

[117]. Nakamura MT, Cheon Y, Li Y, Nara TY. Mechanisms of regulation of gene expression by fatty acids. Lipids. 2004; 39:1077–1083. [PubMed: 15726822]

[118]. Sugden MC, Holness MJ. Mechanisms underlying regulation of the expression and activities of the mammalian pyruvate dehydrogenase kinases. Arch Physiol Biochem. 2006; 112:139–149. [PubMed: 17132539]

[119]. Kersten S, Mandard S, Escher P, Gonzalez FJ, Tafuri S, Desvergne B, Wahli W. The peroxisome proliferator-activated receptor alpha regulates amino acid metabolism. Faseb J. 2001; 15:1971–1978. [PubMed: 11532977]

[120]. Sheikh K, Camejo G, Lanne B, Halvarsson T, Landergren MR, Oakes ND. Beyond lipids, pharmacological PPARalpha activation has important effects on amino acid metabolism as studied in the rat. Am J Physiol Endocrinol Metab. 2007; 292:E1157–1165. [PubMed: 17164430]

[121]. Makowski L, Noland RC, Koves TR, Xing W, Ilkayeva OR, Muehlbauer MJ, Stevens RD, Muoio DM. Metabolic profiling of PPARalpha−/− mice reveals defects in carnitine and amino acid homeostasis that are partially reversed by oral carnitine supplementation. Faseb J. 2009; 23:586–604. [PubMed: 18945875]

[122]. Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metab. 2009; 9:327–338. [PubMed: 19356714]

[123]. Seyfried BT, Kiebish M, Marsh J, Mukherjee P. Targeting energy metabolism in brain cancer through calorie restriction and the ketogenic diet. J Cancer Res Ther. 2009; 5(Suppl 1):S7–15. [PubMed: 20009300]

[124]. Rodriguez JC, Gil-Gomez G, Hegardt FG, Haro D. Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. J Biol Chem. 1994; 269:18767–18772. [PubMed: 7913466]

[125]. Cullingford TE, Dolphin CT, Sato H. The peroxisome proliferator-activated receptor alpha-selective activator ciprofibrate upregulates expression of genes encoding fatty acid oxidation and ketogenesis enzymes in rat brain. Neuropharmacology. 2002; 42:724–730. [PubMed: 11985831]

[126]. Rebrin I, Kamzalov S, Sohal RS. Effects of age and caloric restriction on glutathione redox state in mice. Free Radic Biol Med. 2003; 35:626–635. [PubMed: 12957655]

[127]. Ali MA, Yasui F, Matsugo S, Konishi T. The lactate-dependent enhancement of hydroxyl radical generation by the Fenton reaction. Free Radic Res. 2000; 32:429–438. [PubMed: 10766411]

[128]. Andersson AK, Ronnback L, Hansson E. Lactate induces tumour necrosis factor-alpha, interleukin-6 and interleukin-1beta release in microglial- and astroglial-enriched primary cultures. J Neurochem. 2005; 93:1327–1333. [PubMed: 15934951]

[129]. Zhou W, Mukherjee P, Kiebish MA, Markis WT, Mantis JG, Seyfried TN. The calorically restricted ketogenic diet, an effective alternative therapy for malignant brain cancer. Nutr Metab (Lond). 2007; 4:5. [PubMed: 17313687]

[130]. Freeman JM, Vining EP, Pillas DJ, Pyzik PL, Casey JC, Kelly LM. The efficacy of the ketogenic diet-1998: a prospective evaluation of intervention in 150 children. Pediatrics. 1998; 102:1358–1363. [PubMed: 9832569]

[131]. Cullingford TE. The ketogenic diet; fatty acids, fatty acid-activated receptors and neurological disorders. Prostaglandins Leukot Essent Fatty Acids. 2004; 70:253–264. [PubMed: 14769484]

[132]. Wheless JW. History of the ketogenic diet. Epilepsia. 2008; 49(Suppl 8):3–5. [PubMed: 19049574]

[133]. Blazquez C, Woods A, de Ceballos ML, Carling D, Guzman M. The AMP-activated protein kinase is involved in the regulation of ketone body production by astrocytes. J Neurochem. 1999; 73:1674–1682. [PubMed: 10501215]

[134]. Blazquez C, Sanchez C, Velasco G, Guzman M. Role of carnitine palmitoyltransferase I in the control of ketogenesis in primary cultures of rat astrocytes. J Neurochem. 1998; 71:1597–1606. [PubMed: 9751193]

[135]. Blazquez C, Sanchez C, Daza A, Galve-Roperh I, Guzman M. The stimulation of ketogenesis by cannabinoids in cultured astrocytes defines carnitine palmitoyltransferase I as a new ceramide-activated enzyme. J Neurochem. 1999; 72:1759–1768. [PubMed: 10098887]

[136]. Edmond J. Essential polyunsaturated fatty acids and the barrier to the brain: the components of a model for transport. J Mol Neurosci. 2001; 16:181–193. discussion 215-121. [PubMed: 11478373]

[137]. Konig B, Koch A, Giggel K, Dordschbal B, Eder K, Stangl GI. Monocarboxylate transporter (MCT)-1 is up-regulated by PPARalpha. Biochim Biophys Acta. 2008; 1780:899–904. [PubMed: 18375207]

[138]. Camarero N, Mascaro C, Mayordomo C, Vilardell F, Haro D, Marrero PF. Ketogenic HMGCS2 Is a c-Myc target gene expressed in differentiated cells of human colonic epithelium and down-regulated in colon cancer. Mol Cancer Res. 2006; 4:645–653. [PubMed: 16940161]

[139]. Wenz T, Diaz F, Spiegelman BM, Moraes CT. Activation of the PPAR/PGC-1alpha pathway prevents a bioenergetic deficit and effectively improves a mitochondrial myopathy phenotype. Cell Metab. 2008; 8:249–256. [PubMed: 18762025]

[140]. Kalaany NY, Sabatini DM. Tumours with PI3K activation are resistant to dietary restriction. Nature. 2009; 458:725–731. [PubMed: 19279572]

[141]. Huang H, Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci. 2007; 120:2479–2487. [PubMed: 17646672]

[142]. Aman MJ, Lamkin TD, Okada H, Kurosaki T, Ravichandran KS. The inositol phosphatase SHIP inhibits Akt/PKB activation in B cells. J Biol Chem. 1998; 273:33922–33928. [PubMed: 9852043]

[143]. Meier R, Thelen M, Hemmings BA. Inactivation and dephosphorylation of protein kinase Balpha (PKBalpha) promoted by hyperosmotic stress. Embo J. 1998; 17:7294–7303. [PubMed: 9857186]

[144]. Liu Q, Sasaki T, Kozieradzki I, Wakeham A, Itie A, Dumont DJ, Penninger JM. SHIP is a negative regulator of growth factor receptor-mediated PKB/Akt activation and myeloid cell survival. Genes Dev. 1999; 13:786–791. [PubMed: 10197978]

[145]. Ramaswamy S, Nakamura N, Vazquez F, Batt DB, Perera S, Roberts TM, Sellers WR. Regulation of G1 progression by the PTEN tumor suppressor protein is linked to inhibition of the phosphatidylinositol 3-kinase/Akt pathway. Proc Natl Acad Sci U S A. 1999; 96:2110–2115. [PubMed: 10051603]

[146]. Backman SA, Stambolic V, Suzuki A, Haight J, Elia A, Pretorius J, Tsao MS, Shannon P, Bolon B, Ivy GO, Mak TW. Deletion of Pten in mouse brain causes seizures, ataxia and defects in soma size resembling Lhermitte-Duclos disease. Nat Genet. 2001; 29:396–403. [PubMed: 11726926]

[147]. Du K, Herzig S, Kulkarni RN, Montminy M. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. Science. 2003; 300:1574–1577. [PubMed: 12791994]

[148]. Koo SH, Satoh H, Herzig S, Lee CH, Hedrick S, Kulkarni R, Evans RM, Olefsky J, Montminy M. PGC-1 promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3. Nat Med. 2004; 10:530–534. [PubMed: 15107844]

[149]. Koh HJ, Arnolds DE, Fujii N, Tran TT, Rogers MJ, Jessen N, Li Y, Liew CW, Ho RC, Hirshman MF, Kulkarni RN, Kahn CR, Goodyear LJ. Skeletal muscle-selective knockout of LKB1 increases insulin sensitivity, improves glucose homeostasis, and decreases TRB3. Mol Cell Biol. 2006; 26:8217–8227. [PubMed: 16966378]

[150]. Selim E, Frkanec JT, Cunard R. Fibrates upregulate TRB3 in lymphocytes independent of PPAR alpha by augmenting CCAAT/enhancer-binding protein beta (C/EBP beta) expression. Mol Immunol. 2007; 44:1218–1229. [PubMed: 16949670]

[151]. Scatena R, Bottoni P, Giardina B. Mitochondria, PPARs, and Cancer: Is Receptor-Independent Action of PPAR Agonists a Key? PPAR Res. 2008; 2008:256251. [PubMed: 18645611]

[152]. Nemoto S, Fergusson MM, Finkel T. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{alpha}. J Biol Chem. 2005; 280:16456–16460. [PubMed: 15716268]

[153]. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature. 2005; 434:113–118. [PubMed: 15744310]

[154]. Hou X, Xu S, Maitland-Toolan KA, Sato K, Jiang B, Ido Y, Lan F, Walsh K, Wierzbicki M, Verbeuren TJ, Cohen RA, Zang M. SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. J Biol Chem. 2008; 283:20015–20026. [PubMed: 18482975]

[155]. Lan F, Cacicedo JM, Ruderman N, Ido Y. SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation. J Biol Chem. 2008; 283:27628–27635. [PubMed: 18687677]

[156]. Jager S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A. 2007; 104:12017–12022. [PubMed: 17609368]

[157]. Li X, Monks B, Ge Q, Birnbaum MJ. Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. Nature. 2007; 447:1012–1016. [PubMed: 17554339]

[158]. Irrcher I, Ljubicic V, Hood DA. Interactions between ROS and AMP kinase activity in the regulation of PGC-1alpha transcription in skeletal muscle cells. Am J Physiol Cell Physiol. 2009; 296:C116–123. [PubMed: 19005163]

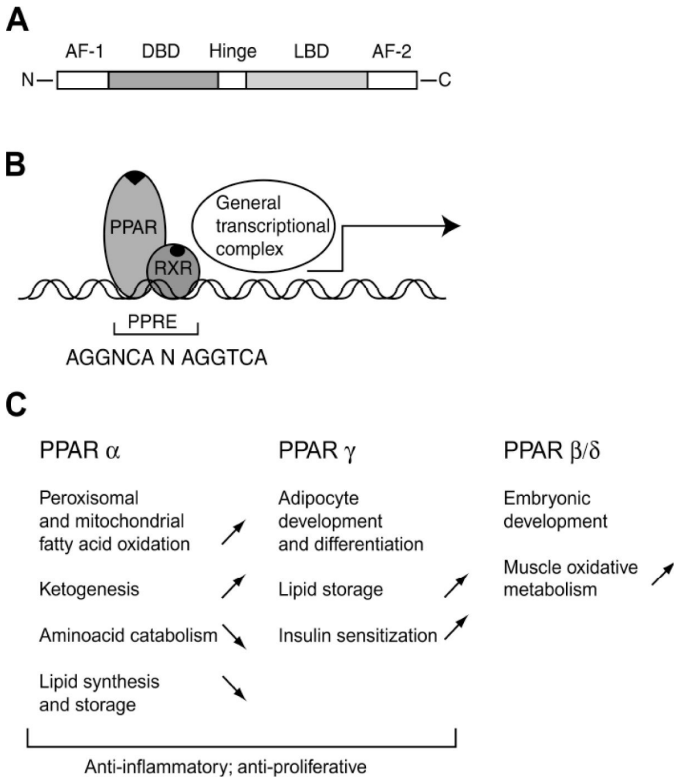

Fig. (1).

Peroxisome proliferator activated receptors (PPARs) general structure and functions. (**A**) PPARs are nuclear receptors and share the same general pattern of structure with other members of the steroid hormone receptor superfamily: N- and C- terminal regulatory regions AF-1 and AF-2 (activation function), respectively, DBD – DNA binding domain with zinc finger motives, LDB – ligand binding domain, hinge region in the middle. (**B**) PPARs act as ligand – activated transcription factors which bind the DR1 consensus sequence together with retinoid X receptors (RXR) [129]. PPAR / RXR heterodimer facilitates the recruitment of other transcriptional activators and the complex including RNA polymerase and general transcription factors. (**C**) Three PPAR isoforms have been identified, each with different physiological areas of activity. The arrows indicate up- or downregulation of the mentioned processes.

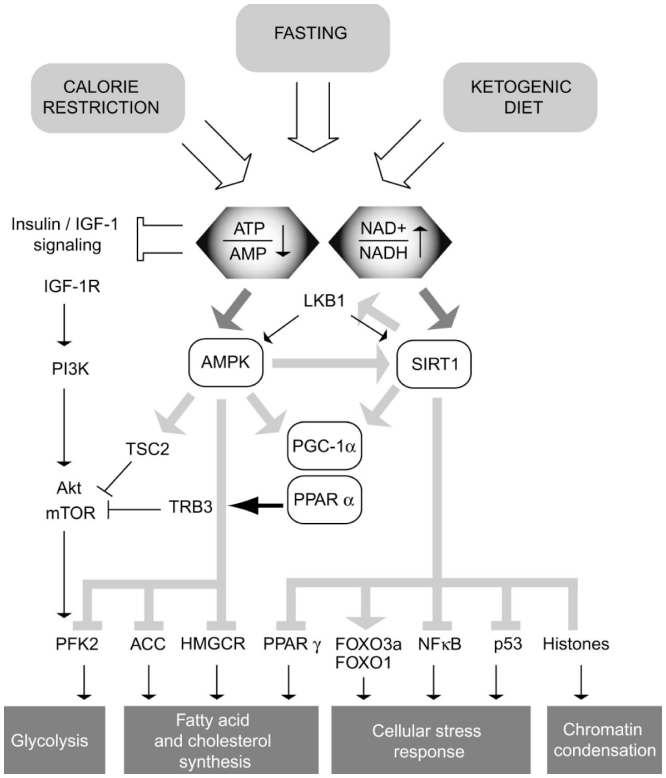

Fig. (2).

Molecular interplay between the proteins activated in response to fasting, calorie restriction and ketogenic diet. Limited availability of nutrients induces the decrease of ATP to AMP ratio, which activates AMP-dependent protein kinase (AMPK) and its downstream effectors; and the increase of NAD^+ to NADH ratio, which activates protein deacetylase SIRT1. AMPK and SIRT1 reprogram the cellular metabolism from high rate of glucose consumption to fatty acid oxidation; simultaneously the anabolic pathways like lipid biosynthesis are downregulated. SIRT1 deacetylates and activates PGC-1α [152, 153]. A positive feedback loop between SIRT1 and AMPK has been reported. A potent AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-riboside (AICAR) induces a strong activation of SIRT1 through the increase of intracellular NAD^+/NADH ratio. This is an indirect process as these proteins do not interact physically and AMPK does not phosphorylate SIRT1 [64]. The activation is reciprocal because SIRT1 deacetylates and activates LKB1, a direct upstream kinase that phosphorylates AMPK on Thr 172 and enhances its activity [154, 155]. Both AMPK and SIRT1 employ PGC-1α as their downstream effector, that is phosphorylated on Ser 538 and Thr 177 by AMPK [156] and deacetylated by SIRT1 [153]. Phosphorylation by AMPK improves PGC-1α stability and activity, in contrast to Akt phosphorylation on Ser 570, which leads to protein destabilization[157, 158]. PGC-1α cooperates with PPAR α in regulation of certain signaling pathways as insulin / IGF-1 / Akt. During the energetic stress, SIRT1 induces FOXO1 and FOXO3a transcription factors. On the epigenetic level, SIRT1 deacetylates histones which leads to formation of transcriptionally silent regions of condensed heterochromatin.

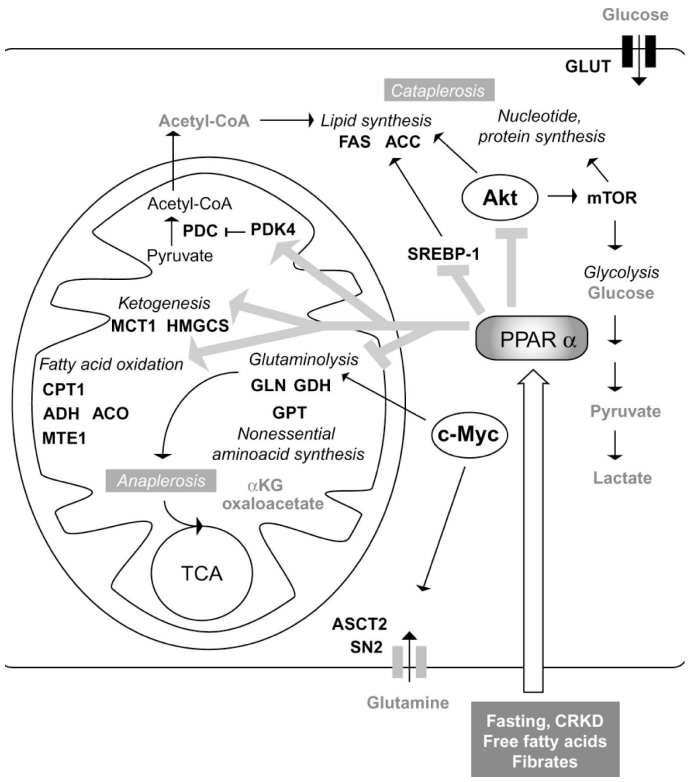

Fig. (3).

PPAR α activation in response to calorie restricted ketogenic diet (CRKD). Endogenous ligands like free fatty acids or drugs from fibrate class may have a therapeutic impact on cancer cell's mitochondrial processes: fatty acid oxidation, ketogenesis, glutaminolysis and pyruvate conversion to acetyl-CoA. PPAR α activity counteracts metabolic changes exerted by oncogenes: c-Myc driving glutaminolysis (anaplerosis) and Akt enforcing aerobic glycolysis and lipid synthesis (cataplerosis). For more details see the text.