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Review

Managing lipid metabolism in proliferating cells: New perspective for metformin usage in cancer therapy

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ABSTRACT

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. The dependence of tumour cells on the deregulated lipid metabolism suggests that proteins involved in this process could be excellent chemotherapeutic targets for cancer treatment. Due to its rare side effects in non-cancerous cells, metformin has been recently revaluated as a potential anti-tumourigenic drug, which negatively affects lipid biosynthetic pathways. In this review we summarised the emerging molecular events linking the anti-proliferative effect of metformin with lipid metabolism in cancer cells.

Contents

1. Introduction

Since Warburg's discovery, in the 1950s, that cancer cells preferentially utilize glycolysis rather than the more efficient oxidative phosphorylation (OXPHOS) to produce ATP [1], more than five decades of research have made it clear that tumour cells present several alterations at the level of metabolic pathways [2]. Overall, these alterations are

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aimed at increasing the incorporation and usage of nutrients, necessary to fulfil their high energetic requirements. The addiction to either glucose or glutamine, or even both, is now a well-established hallmark of many tumour cells. Indeed, glutamine and glucose are two extremely versatile molecules which can provide cancer cells not only ATP but also substrates for the synthesis of macromolecules (proteins, nucleic acids, lipids) and reducing equivalents (NADPH). The higher dependence of cancer cells to a continuous supply of nutrients with respect to non-transformed cells opens the possibility for cancer therapies which targets tumour-specific metabolic network. Indeed, many molecules and drugs have been developed and tested so far, with some of them that have reached clinical trial phases [3]. The demonstration that nutrient incorporation by cancer cells greatly exceeds the requirement of ATP, reveals that the production of biomass could be even more crucial than the synthesis of ATP in sustaining cancer cell proliferation and growth [4]. In this context, along with the key role of glucose and glutamine, a growing body of evidence is emerging about the deep involvement of lipid metabolism in tumours. Lipid metabolism, particularly that regarding fatty acids (FAs), is tightly linked to those of glucose and glutamine, since both fuel FA synthesis by continuously providing substrates such as NADPH and acetyl-CoA. *De novo* synthesis of FAs in adult normal tissues mainly occurs in the liver and in the adipose tissue. However, several studies revealed that tumour cells reactivate lipid neo-synthesis, making a high degree of FA synthesis, defined as lipogenesis, a key metabolic footprint of nearly all cancers that is required for both tumourigenesis and cancer progression [5–9]. An enhanced expression of lipogenic genes has been found in association with several types of cancers with aggressive phenotypes [10,11]. The function of FAs in tumour cells is not only limited to their well-known role as storage of energy. Indeed, like other macromolecules, energy production is only one, and probably not the most important, aspect of lipid contribution in sustaining tumour growth. Other crucial roles that lipids exert within the cells include: *i*) supply of building blocks for membrane biosynthesis; *ii*) second messengers and signalling molecules; and *iii*) involvement in post-translational modifications of proteins. Considering the role of FAs in many cellular functions, targeting enzymes involved in or related to their metabolism may be a feasible and winning strategy for preferentially targeting cancer cells, with a reduced side-effect for normal cells and the entire organism. In this review we provide an overview of the main alterations associated with lipid metabolism and the current advances in the development of anti-cancer strategies, which target lipid-related pathways. Particular attention will be directed to the emerging role of an old anti-diabetic drug metformin, as a promising anti-cancer therapeutic treatment.

2. Tumour-specific dysregulation of lipogenesis in cancer cells

The exacerbated lipogenesis in cancer cells is not only caused by the up-regulation of lipid metabolising enzymes, but is also directly coupled to other common metabolic pathways such as those related with glycolytic or glutaminolytic flux (Fig. 1). In particular, glucose meets lipid synthesis into mitochondria at the point of citrate, an intermediate of the Krebs cycle. During aerobic glycolysis, glucose carbons are funnelled into the mitochondria as pyruvate leading to an increase in the mitochondrial concentration of citrate. In highly proliferating cells, mitochondrial citrate is exported to the cytosol via the tricarboxylate transporter, wherein citrate is used as a biosynthetic precursor for lipogenic pathways. Citrate is cleaved by ATP-citrate lyase (ACLY) to generate acetyl-CoA and oxaloacetate. Cytosolic oxaloacetate is reduced to malate, which can then return to the mitochondria, recycling carbon and shuttling reducing equivalents into the mitochondria [12]. The conversion of cytosolic oxaloacetate to malate is driven by the relatively high cytosolic NADH/NAD^+ ratio present in glycolytic cells [13,14]. Malate can enter the mitochondrial matrix and be converted to oxaloacetate to complete the substrate cycle. In parallel, acetyl-CoA represents the start-up molecule for newly synthesized lipids [15]. In cellular compartment a well-organized enzymatic structure is engaged to metabolize carbons from cytosolic citrate to FAs. ACLY, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and sterol regulatory element-binding proteins (SREBPs) are the best characterized metabolic checkpoints involved in the progression of cancer development and for which several pharmacological approaches are under evaluation. Hereinafter, these pivotal components of lipid metabolism will be reviewed.

2.1. ACLY

Proliferating cells require lipid building blocks for membrane formation. It has long been established that most normal tissues obtain the bulk of their required lipids from the diet and the circulation. However, many human tumours meet this need in a largely self-sufficient manner by overexpression of several lipogenic enzymes and activation of lipogenic pathways [16]. One of these enzymes is ACLY, a homotetrameric enzyme that links glucose with lipid metabolism by shuttling metabolites from the glycolysis and the citric acid cycle to the FAs and cholesterol synthesis pathways by converting cytosolic citrate into oxaloacetate and acetyl-CoA, which is the key building block for *de novo* lipogenesis. High levels of glucose and growth factors (*i.e.* insulin/insulin-like growth factor-1 (IGF-1)) activate PI3K/Akt/mTOR oncogenic pathways promoting cancer progression, consequentially to ACLY induction [17–20]. It thus becomes evident that signalling pathways that contribute to a glycolytic phenotype and play an important role in tumourigenesis can also lead to increased ACLY levels and/or activity. These de-regulated pathways may partly account for the evidence that ACLY activity is found to be significantly elevated in lung, prostate, bladder, breast, liver and stomach tumours [21–23]. Interestingly, by using Oncomine and unbiased proteomic profiling, it has been found that ACLY was up-regulated in colorectal cancer compared with its levels in normal mucosa. Moreover, overexpression and activation of ACLY were found to be statistically significant negative prognostic factors for at least lung and colon cancers [22,24]. Furthermore ACLY acetylation at three lysine residues (Lys 540, 546 and 554) is increased in human lung tumours. Indeed it has recently been demonstrated that acetylation of these residues enhances ACLY activity by preventing its ubiquitination and degradation, resulting in increased FA biosynthesis and tumour cell growth under high glucose conditions [25].

2.2. ACC

ACC is a rate-limiting enzyme in *de novo* FA synthesis, catalysing the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA. Malonyl-CoA is a substrate of FAS for acyl chain elongation and an inhibitor of carnitine palmitoyltransferase I (CPT-I) for FA β-oxidation. ACC is positively and allosterically regulated by citrate and glutamate and negatively and allosterically regulated by long- and short-chain fatty acyl-CoAs such as palmitoyl-CoA. There are two isoforms of ACC, namely ACC1 and ACC2, which, although encoded by separate genes, exhibit considerable sequence identity and have the same domain structure responsible for enzyme activity. However, ACC1 seems to be the limiting enzyme in proliferating cancer cells [26]. This could be related with an unlike biochemical role of ACC1 and ACC2. In particular, ACC1 is functional to regulate FA synthesis whereas ACC2 mainly regulates FA oxidation and similarly to ACLY, ACC1 is under the insulin/IGF-1 signal transduction pathway [27]. ACC1 has been found up-regulated in proliferating cancer cell lines such as prostate, breast and liver. Indeed, it has been shown that knock-down of ACC1 by siRNA promotes apoptosis in prostate cancer and breast tumour cells but not in control noncancerous cells, underlining cancer cells' higher reliance on this enzyme than normal tissues [28,29]. ACC1 is up-regulated in breast cancer cell lines overexpressing the tumour aggressiveness marker Human Epidermal Growth Factor Receptor 2 (HER2), with respect to breast cancers with low or normal levels of the receptor. HER2 enhances ACC1 expression

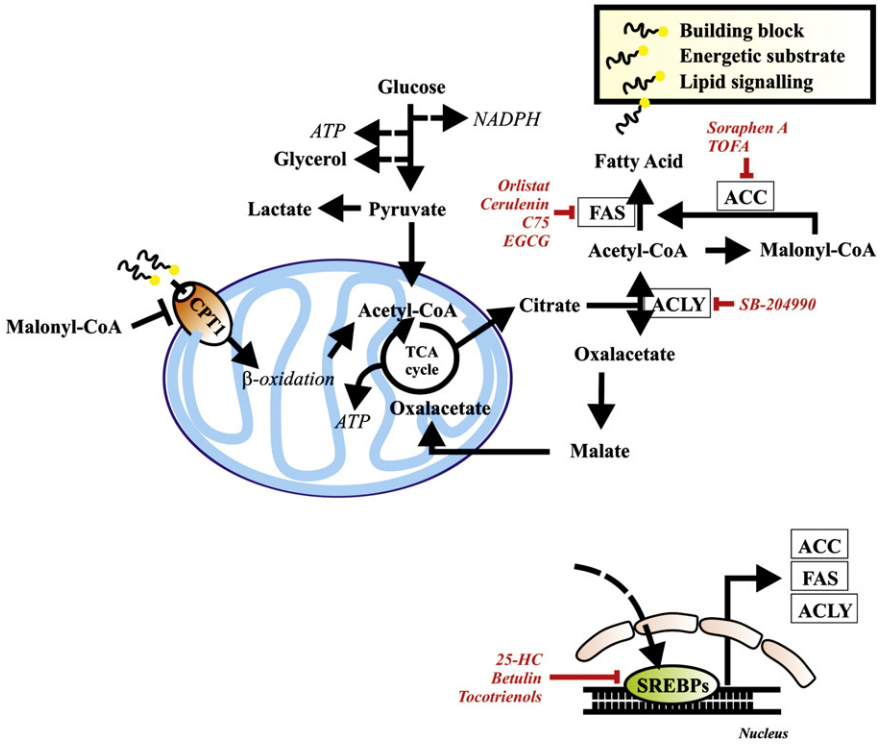

Fig. 1. Lipid metabolism in proliferating cancer cells. Mitochondrial citrate can be synthesized when acetyl-CoA condenses with oxaloacetate (OAA) *via* the activity of citrate synthase. Mitochondrial citrate is exported to the cytosol and cleaved by ATP citrate lyase (ACLY) to generate acetyl-CoA and oxaloacetate. Acetyl-CoA is carboxylated in malonyl-CoA by acetyl-CoA carboxylase (ACC). Malonyl-CoA is a substrate of fatty acid synthase (FAS) for acyl chain elongation. Sterol retinol element binding proteins (SREBPs) are factors involved in the transcriptional controls of ACLY, ACC and FAS. In red are reported the main lipogenic enzyme inhibitors.

at translational level by the activation of the PI3K/AKT/mTOR pathway [30].

2.3. FAS

FAS is the sole lipogenic enzyme in the human genome capable of the reductive *de novo* synthesis of long-chain FAs from acetyl-CoA, malonyl-CoA, and NADPH. FAS produces the 16-carbon saturated palmitate, predominantly in the liver and other lipogenic tissues for the synthesis of triacylglycerol as storage fuel molecule. However, it has been reported that the predominant fate of the palmitate in cancer cells is the esterification to lipid-related membrane structures such as phospholipids and sphingolipids. Increased levels of FAS have emerged as a typical phenotype of most human carcinomas. Furthermore, a raised FAS expression is an indicator of poor prognosis in breast and prostate cancers, with respect to other tumours. Indeed it has been found that tumours overexpressing FAS are often associated with resistance to some anticancer drugs, in particular DNA-damaging molecules such as gemcitabine and doxorubicin [31]. The exact mechanisms of FAS-induced drug resistance are not clear yet, although one proposed mechanism comes from the observation that FAS up-regulates Poly (ADP-ribose) polymerase-1 (PARP-1), an enzyme involved in the repair of DNA damages, thus dampening the cytotoxic effects of DNA damaging drugs.

2.4. SREBPs

SREBPs, which are a target of PI3K/Akt (under insulin/IGF-1 signalling cascade), are transcription factors of the helix–loop–helix leucine zipper family strongly involved in the control of lipid biogenesis. SREBPs are expressed as inactive precursors and reside as integral transmembrane proteins within the ER membrane, where they bind to the SREBP cleavage activating protein (SCAP). There are three SREBP isoforms generated by alternative splicing such as SREBP1a, SREBP1c and SREBP2 and, although there is an overlap between their target genes, SREBP1 mainly regulates FAs, phospholipid and triacylglycerol synthesis. By contrast, SREBP2 controls the expression of cholesterol-synthesis genes. Amongst SREBP target genes, which are generally up-regulated in cancerous cells and in several human tumours, FAS, ACC and ACLY are included [12]. The idea of an indirect targeting of lipid metabolism, by means of transcriptional regulators of lipogenic enzymes, could be a winning one for anti-cancer therapy, and SREBPs could represent the best candidates, even if this approach remains up to date little explored. For instance, it has been shown that SREBP-1/2 inhibition by 25-hydroxycholesterol (25-HC) induces cell death in epidermal growth factor receptor (EGFR) expressing glioblastoma cells [32]. Moreover, in a very recent paper, Williams and colleagues demonstrated that the loss of SREBP activity inhibits cancer cell growth and viability by uncoupling fatty acid synthesis from desaturation [33]. As far as cholesterol biosynthesis is concerned, natural compounds such as betulin and tocotrienols can efficiently target SREBP-2, as they decreased prostate cancer cells' survival [34].

3. Lipid catabolism constrains cancer cell proliferation: ATGL and Lipa

Although biosynthetic pathways can generate lipids, it is conceivable that cancer cells living in glucose-restricted conditions catabolize stored lipids to maintain energetic homeostasis [35]. In this regard it has been demonstrated that FAs liberated from particular cellular structures, such as lipid droplets (LDs), are efficiently used to cope with energy demand of nutritionally stressed cells [35,36]. LDs are surrounded by several proteins, which are involved in the removal of FAs from stored triacylglycerols (TGs) [37]. Amongst these proteins, adipose triglyceride lipase (ATGL) seems to have a key role [38]. Recently it has been reported that ATGL up-regulation is operative to counteract energetic catastrophe funnelling FAs from LDs towards mitochondrial oxidation [36]. Similarly, also cytosolic phospholipase A2 (cPLA2) mediates mitochondrial β-

oxidation in nutrient restricted cancer cells [35]. However, concomitantly to cytosolic lipolysis, it is emerging that lipids can be also catabolized through autophagy-mediated lipolysis termed lipophagy [39–42]. Lipophagy is an essential, conserved lysosomal degradation pathway that controls the TG degradation to maintain cellular energetic levels [40]. The main mediator involved in lipid catabolism by lipophagy is lysosomal acid lipase (Lipa), which is markedly up-regulated upon nutrient restriction [39,42]. In particular HepG2 cell lines as well as *in vivo* models, display a concomitant up-regulation of Lipa and LC3 [39]. In an opposite manner the down-regulation of key autophagy proteins is associated with intracellular lipid accumulation in cancer cells [43]. Similarly, the lack of Lipa in nutrient restricted cells promotes energetic catastrophe [42].

In particular, activated FAs (FA-CoAs) are degraded by mitochondrial β-oxidation, producing acetyl-CoA and reducing equivalents for oxidative phosphorylation [44]. The mode of regulation of β-oxidation ensures that lipid synthesis and degradation are mutually exclusive [5]. Indeed, β-oxidation is increased when ACC is inhibited because of the depletion of malonyl-CoA [45]. Interestingly, the reduction of cancer cell proliferation by inhibiting ACC may also be due in part to an increased catabolism of FAs [46]. It has been reported that treatment of cancer cells with drugs enhancing lipid catabolism lowers cell proliferation rate [47,48]. Conceivably, increased lipid catabolism via oxidation could lead to a reduction in FAs available for use as membrane building blocks or signalling lipids thus constraining cancer cell proliferation.

4. Anticancer strategies targeting lipid metabolism

The discovery that tumour cells rely on *de novo* synthesis of FAs and lipids to form new membranes thus sustaining high proliferation rate, has paved the way to a field of research aimed to target lipogenic enzymes and their regulators. To date, research has focused on the main enzymes involved in FA synthesis (the abovementioned ACLY, ACC, and FAS are doubtlessly the best target candidates) and their transcriptional positive regulators (*i.e.* SREBPs). Many inhibitors of these enzymes and regulators have been developed and tested both in cell lines and animal models, however, to our best knowledge, none of them has reached yet clinical trial phases. In the following part the most promising inhibitors of lipogenic pathways in cancer cells will be reviewed.

4.1. SB-204990

ACLY is an appealing target for anti-cancer therapy because of its strategic position in lipid synthesis pathways, providing acetyl-CoA as substrate for the generation of not only FAs, but also cholesterol and isoprenoids. Inhibition of the enzyme by means of siRNA or stable shRNA showed positive results in terms of anti-neoplastic activity both in *in vitro* and *in vivo* models [17,49], and this prompted research to focus on SB-204990, a chemical specific inhibitor of ACLY [50]. SB-204990 inhibits proliferation of tumour cell lines A549, PC3 and SKOV3, and exerts cytostatic effect in xenografts A549 and PC3 cell lines in nude mice [49]. However, less anti-neoplastic effects are observed in SKOV3 xenografts wherein addition of SB-204990 leads to a consistent loss of weight in animals. This is probably due to the lower reliance of this tumour histotype on aerobic glycolysis, with respect to the other two cell lines. Indeed, with ACLY being the bridge between glycolytic metabolism and lipid synthesis, it is likely that its inhibition has the best anti-tumour effects on high glycolytic cancer cells.

4.2. ACC inhibitors

The finding that RNA interference of ACC1 strongly inhibited tumour cell growth and induced cell death [29] puts this enzyme in the spotlight as a possible target for anticancer therapy. Soraphen A is a natural compound known to inhibit activity of both isoforms of ACC at nanomolar concentration [51], by binding an allosteric site. Soraphen A decreased FA synthesis, inhibited cell proliferation and induced cell death in high lipogenic prostate cancer cells [52]. TOFA (5-(tetradecyloxy)-2-furancarboxylic acid) is a chemically synthesized allosteric inhibitor of ACC. It has been shown that lung cancer cells NCI-H460 and colon carcinoma cells HCT-8 and HCT-15 treated with TOFA underwent reduced FA synthesis and subsequent apoptotic cell death [53]. Interestingly TOFA-induced cell death was completely reverted by adding palmitic acid to the cells, indicating that FA depletion leads to cell death. Despite these promising results, some contradictory findings still put in doubt the real efficacy of inhibiting ACC as a therapeutic strategy. For instance, it has recently been shown that ACC knockdown promotes the survival of different tumour cell lines, under energetic stress conditions, by means of maintaining redox balance [54].

4.3. FAS inhibitors

FAS is doubtless the most attractive and studied metabolic enzyme as far as therapeutic targeting of lipid metabolism in tumours is concerned. The reason should be searched in the evidence that many human tumours overexpress FAS and tightly depend on this enzyme for FA biosynthesis, with respect to normal tissues, which could utilize also exogenous dietary FAs [55]. Therefore it's not surprising that many compounds have been developed to inhibit FAS thus depleting cancer cells of their major source of FAs. Orlistat that was developed as an anti-obesity drug is an efficient irreversible inhibitor of FAS as well. Orlistat exerts anti-cancer effects both in *in vitro* and *in vivo* models of different tumour histotypes (prostate, gastric, melanoma, breast) [56–60], however its use still has some pharmacological limitations due to low solubility, cell permeability and poor selectivity. Another class of FAS inhibitors is small molecule cerulenin and its analogue C75, both of which have shown potent anti-tumour activity. Cerulenin triggers apoptotic death in breast cancer cell lines and delays tumour progression in ovarian cancer xenografts [61,62]. Although being a less efficient FAS inhibitor, C75 is able to induce apoptosis, block FA synthesis and inhibit tumour development in an *in vivo* breast cancer model [63]. However, reduced food intake and body weight observed upon administration of these two FAS inhibitors are side effects that can't be neglected [64]. In order to overcome these limitations new drugs with better pharmacological properties have been conceived, one for all is C93, which inhibits tumour growth both *in vitro* and *in vivo* without causing body weight loss [58,65]. Finally, many plant-derived natural compounds, belonging to the class of polyphenols (epigallocatechin-3-gallate EGCG) and flavonoids (luteolin, taxifolin, kaempferol, apigenin and quercetin), have been found to efficiently inhibit FAS [60]. Amongst them is EGCG, which can be found in green tea and is the best characterized, and even if it can target different cellular pathways, its pro-apoptotic properties seem to be ascribed to its inhibitory activity of FAS [66].

5. Undisclosed roles of AMPK-mediated metformin effects on cancer lipid metabolism

Recently, a renewed interest in the potential involvement of Adenosine Monophosphate-activated Protein Kinase (AMPK) in the control of cancer cell growth and proliferation has been reported [67]. AMPK is chiefly known as the main cellular energy sensor and actively participates in the control of cancer cell metabolism and several cellular processes, ranging from proliferation to autophagy and apoptosis [68,69]. In cancer cells, the activation of this kinase down-regulates anabolic processes promoting cell cycle arrest [69]. The role of AMPK in tumours mostly stems from the observation that germline mutations of the tumour suppressor liver kinase B1 (LKB1), the major upstream activator of AMPK, are the cause of the Peutz–Jeghers Syndrome, a condition

that increases the risk of developing pancreatic, gastrointestinal, breast, and non-small cell lung cancers (NSCLC) [70]. This evidence, together with the inverse relationship between AMPK phosphorylation levels with histological grade and axillary node metastasis of primary breast tumours strengthens the role of this kinase as tumour suppressor and keeper of cellular homeostasis, suggesting that AMPK activation/re-activation could be a potentially striking strategy for therapeutic purposes.

5.1. AMPK controls lipid metabolism-related pathways in cancer cells

It has been widely demonstrated that phospho-activated AMPK (AMPKpT172) efficiently inhibits ACC by selective phosphorylation [71]. Furthermore, AMPK suppresses the proteolytic processing and the transcriptional efficiency of lipogenic transcription factors SBREPs, in particular SREBP1c [72,73]. The latter point strongly suggests that AMPK can indirectly inhibit all the lipogenic enzymes so far cited (ACC, FAS, ACLY) by means of the suppression of the activity of their main activating transcription factor (*i.e.* SREBP1c). For instance it has been shown that AMPK reduces the expression levels of FAS in liver cells, and this effect is ascribed to AMPK-mediated inhibition of SREBP1c [74–76]. Another important point through which AMPK activation could limit cancer cell growth is its ability to inhibit mammalian target of rapamycin (mTOR) [77–79]. mTOR upregulates energy-consuming cellular processes and controls cell growth as well as cancer cell proliferation. mTOR, target of PI3K/Akt (downstream of insulin/IGF-1), plays a central role in the metabolic cellular control. In particular, it activates lipogenesis processes through the induction of SREBP1c and FAS thus supplying lipid building blocks in proliferating cancer cells [80,81]. Porstmann et al. observed that inhibition of mTOR complex 1 (mTORC1) with rapamycin blocks Akt-induced SREBP-1c nuclear localization, the expression of lipogenic genes, and the production of various classes of lipids (unsaturated and saturated fatty acids, phosphatidylcholine, and phosphatidyl-glycerol) [81]. The knockdown of Raptor (a key component of mTORC1), but not Rictor (functional to mTOR complex 2 (mTORC2)), showed similar effects, indicating that SREBP-1 activation by Akt depends on mTORC1 but not on mTORC2. This finding supports previous works that showed that rapamycin reduces the expression of many SREBP-1 target genes, ACC and FAS [82,83]. Furthermore, the inhibition of mTOR pathway has been identified as a mechanism by which AMPK regulates autophagy [77]. Several studies have confirmed that activation of autophagy is essential to preserve cellular viability during nutritional deprivation, and that mutants defective in autophagy were causative of energetic catastrophe [84]. A plethora of studies indicates that protein degradation by autophagy is the main source of energy. However, the autophagy-provided amino acids are a relatively inefficient substrate for energy production. Recent studies support that autophagy can also provide more energetically-rich molecules, such as FAs. It is conceivable that autophagy-released FAs could be funnelled towards mitochondria for their oxidation [85]. Finally, another pathway through which AMPK could modulate cancer cell progression is its ability to inhibit FA release from adipose cells [42,86,87]. Indeed, even if cancer cell progression is mainly maintained by *de novo* synthesis of lipids, adipose cells delivering FAs could promote a favourable milieu for their proliferation. It has been reported that AMPK inhibits lipolytic signal cascade in adipose cells by phosphorylating hormone sensitive lipase (HSL) on serine 565 (HSLpS565) [88]. At this point a persistent activation of AMPKpT172-HSLpS565 axis could limit the availability of FAs to tumours. Interestingly, adaptive responses up-regulating FA transporters have been identified in cancer cell types typically metastasising in lipid-rich tissues [89–91]. The findings above-described strongly suggest that active AMPK could limit cell cycle progression in proliferating cells through several checkpoint delivering membrane-building substrates such as lipids.

5.2. Metformin: an old drug for a novel clinic approach

Amongst drugs activating AMPK, well-defined is the role of metformin. Metformin belongs to the family of biguanide and is the most widely used anti-diabetic drug in the world [92,93]. Encouraging results emerged from studies indicating that metformin can potentially be used as an efficient anticancer drug in various neoplasms such as prostate, breast, lung and pancreas cancers [94–97]. These results were confirmed by retrospective epidemiological studies that reported a reduction in cancer risk in diabetic patients treated with metformin [98,99]. Similarly, retrospective and observational studies reported reduced incidence of neoplastic diseases and cancer mortality in type 2 diabetes patients treated with metformin [100–102]. These observations are consistent with *in vitro* and *in vivo* studies showing antiproliferative action of metformin on various cancer cell lines and several cancers in animal models [103,104]. Recently, the protective effects of metformin or phenformin, a more efficient liposoluble analogue, were investigated in cancer-prone mouse model. Phosphatase and tensin homolog $(PTEN)^{+/-}$ mice spontaneously develop tumours, particularly lymphomas, and the onset of tumours occurred even earlier when they were crossed with a strain that had mutations that caused reduced expression (*i.e.*, were hypomorphic) for LKB1. Treatment of $PTEN^{+/-}$ mice with metformin or phenformin, significantly delayed the onset of tumour formation, and was associated with increased AMPK activity [105]. In particular, it has been demonstrated that phenformin selectively promoted apoptosis in LKB1-deficient NSCLC cells [106]. Indeed, although several pathways have been defined about the anti-cancer properties of metformin, AMPK-mediated signal transduction cascade seems to be the master site of action [107]. Indeed, AMPK knockdown by siRNA or AMPK inhibitors partially reverts the anti-proliferative action of metformin in breast and ovarian cancer cells [108–110]. It has been also demonstrated that AMPK activation either by metformin or phenformin also inhibits mTOR axis, which governs several anabolic pathways (*i.e.* lipogenesis), inducing autophagic cell-death in melanoma and leukaemia cancer cells [77,78]. Overall these data suggest that the effectiveness of metformin in limiting cancer cell development could be strongly related to its ability to impact lipid metabolism via AMPK activation. However, the mechanism by which metformin activates AMPK in cells is still debated. It is now thought that the pleiotropic effects of metformin or phenformin originate from the primary actions of the drug on the mitochondria, where it inhibits oxidative phosphorylation (at respiratory complex I) in a manner that has not yet been described in detail [111,112]. The known consequence of mitochondrial complex I inhibition is the decline of mitochondrial ATP generation, but there is also evidence for altered redox status associated with an increase in reactive oxygen species (ROS) production generating a mild oxidative stress [113]. Although AMPK could be the primary therapeutic target of metformin, one issue that is not completely resolved concerns the high concentrations of the drug (typically 1–10 mM) that are required to activate AMPK in cultured cells. These are strongly higher than the concentrations estimated to occur in human plasma (10–40 μM) following a therapeutic dose of around 30 mg/kg of metformin [114]. This apparent discrepancy might be explained by the lack of expression of organic cation transporter-1 (OCT1) in many cultured cell lines [114]. Interestingly, it has been reported that the down-regulation of OCT1 observed in some tumours is related with cancer development [115]. Knockdown of OCT1 reduced sensitivity of epithelial ovarian cancer cells to metformin, but interestingly not to another biguanide, phenformin, with respect to both activation of AMPK and inhibition of proliferation [116]. Indeed, although phenformin has also been reported to be transported by OCT1, its greater hydrophobicity and more potent activity in a variety of cultured cells indicate that it can get its intracellular target, independently of OCT1 transporter [116,117]. These results suggest that there may be settings where drug uptake limits direct action of metformin on neoplastic cells, raising the possibility that metformin may not be the optimal biguanide for clinical investigation (Fig. 2).

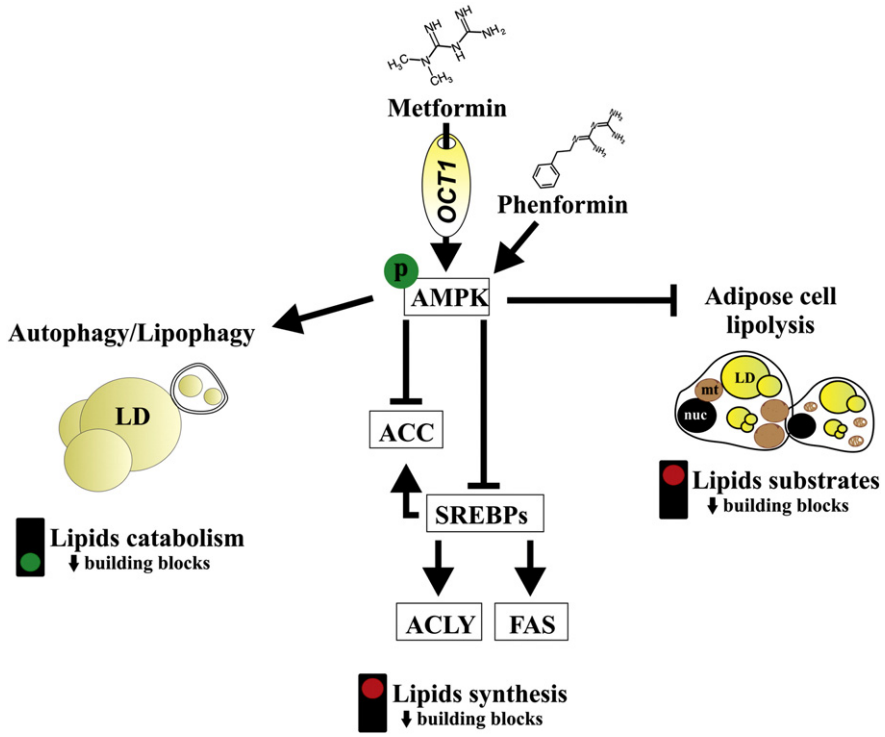

Fig. 2. Biguanide-mediated AMPK activation and its impact on lipid metabolism. Metformin or phenformin limits lipogenic enzyme efficiency enhancing lipid breakdown by AMPK activation. The concomitant lipogenic enzyme inhibition and lipid catabolism reduce the lipid building block availability restraining cancer cell proliferation. Biguanides also reduce the fatty acid release from adipose tissue thus limiting the lipid delivery from extracellular milieu.

6. Emerging evidences describing the AMPK-independent effects of metformin in cancer

Nowadays it is emerging that metformin acts to modulate lipid metabolism in an AMPK-independent manner [118]. Recently, the AMPK activation in the liver of metformin-treated mice has been reported. This is in line with several recent studies highlighting AMPK-independent effects of metformin on tumour growth *in vitro* and in *vivo* [118–121]. In hepatocarcinoma cell lines, metformin was effective to dampen cell proliferation concomitantly to a decreased level of several lipogenic enzymes such as ACC, ACLY and FAS. Additionally, restoring lipogenic gene expression by ectopic expression of the lipogenic transcription factor SREBP1c rescues metformin mediated growth inhibition [122]. Other studies show that metformin treatment significantly inhibited proliferation of diverse chemo-responsive and -resistant ovarian cancer cell lines, and caused cell cycle arrest accompanied by decreased cyclin D1 and increased p21 protein expression. However, although an activated AMPK form was revealed in various metformin-treated ovarian cancer cell lines, this drug negatively affected cell proliferation and lipid biosynthetic pathways also in AMPK silenced ovarian cancer cells. A well-characterized pathway is the inhibition of mTORC1 independently of the activation of the energy sensor AMPK. In particular, metformin suppress mTORC1 by inhibiting Rag GTPases-mediated signalling events [118]. Recently it has been also showed that antiproliferative action of metformin in prostate cancer cell lines is mainly mediated by the up-regulation of REDD1 (regulated in development and DNA damage responses 1), a negative regulator of mTORC1. Indeed, REDD1 inhibition reverses metformin-induced cell-cycle arrest and significantly protects from the effects of metformin on cancer cell proliferation. Furthermore, metformin was reported to decrease the expression of the oncoprotein HER2 (*erbB-2*) in human breast cancer cells *via* a direct and AMPK-independent inhibition of p70-S6 Kinase 1 (p70S6K1) (a downstream of mTORC1 signalling pathway) activity [123]. These data highlight a role of metformin as an antiproliferative therapeutic drug that can act through both AMPK-dependent as well as AMPK-independent pathways.

7. Concluding remarks

A high membrane remodelling characterizes proliferating cells, as cancer cells. Lipids represent the main building block precursors of these cellular structures. It is widely accepted that the ability of cancer cells to synthesize lipids is strongly related with their greed for glucose. In doing so, cancer cells engage key lipogenic enzymes that are generally up-regulated in aggressive tumours. Relative to these aspects several drugs targeting lipogenic enzymes were designed. Side effects in non-cancerous cells have been, however, reported. This leads to the search of novel non-toxic anticancer drugs. In this scenario, a role for metformin in cancer therapy is emerging. One of the advantages of metformin is its relatively safe toxicity profile. In addition, metformin is already approved for diabetes and therefore its introduction into the clinic is streamlined [122]. There are currently about 20 clinical trials investigating the usage of metformin in cancer therapy (ClinicalTrials.gov:http://clinicaltrials.gov/ct2/results?term=metformin+cancer&pg=1). However, even if the mechanisms by which metformin affects cancer development are not yet completely clarified, a potential action could be related to its ability to impact on lipid metabolism. The molecular transducer that mediates the metformin properties has been identified in AMPK. AMPK negatively affects lipid availability for building cell membrane by *switching off* lipogenic enzymes and cancer-adipose tissue cross talk and, at the same time by *switching on* lipid catabolism for energy supply. The reported anticancer activity of metformin or more lipophilic analogues such as phenformin, could be identified as emerging low-toxic drugs counteracting tumour progression by managing lipid building block in proliferating cancer cells.

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