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## Review

# Metformin: Taking away the candy for cancer?

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## ABSTRACT

Metformin is widely used in the treatment of diabetes mellitus type 2 where it reduces insulin resistance and diabetes-related morbidity and mortality. Population-based studies show that metformin treatment is associated with a dose-dependent reduction in cancer risk. The metformin treatment also increases complete pathological tumour response rates following neoadjuvant chemotherapy for breast cancer, suggesting a potential role as an anti-cancer drug. Diabetes mellitus type 2 is associated with insulin resistance, elevated insulin levels and an increased risk of cancer and cancer-related mortality. This increased risk may be explained by activation of the insulin- and insulin-like growth factor (IGF) signalling pathways and increased signalling through the oestrogen receptor. Reversal of these processes through reduction of insulin resistance by the oral anti-diabetic drug metformin is an attractive anti-cancer strategy. Metformin is an activator of AMP-activated protein kinase (AMPK) which inhibits protein synthesis and gluconeogenesis during cellular stress. The main downstream effect of AMPK activation is the inhibition of mammalian target of rapamycin (mTOR), a downstream effector of growth factor signalling. mTOR is frequently activated in malignant cells and is associated with resistance to anticancer drugs. Furthermore, metformin can induce cell cycle arrest and apoptosis and can reduce growth factor signalling. This review discusses the role of diabetes mellitus type 2 and insulin resistance in carcinogenesis, the preclinical rationale and potential mechanisms of metformin's anti-cancer effect and the current and future clinical developments of metformin as a novel anti-cancer drug.

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## 1. Introduction

In 1924, the Nobel laureate Otto Heinrich Warburg first hypothesised the existence of a connection between cellular metabolism and malignancy.<sup>1</sup> The signalling pathways controlling metabolism and cancer and their interactions are now being unravelled and evidence is accumulating that con-

ditions associated with metabolic disturbances, such as diabetes mellitus type 2, increase cancer risk and adversely influence cancer prognosis.

AMP-activated protein kinase (AMPK) is a central cellular energy sensor which may be a crucial factor in the interaction between metabolism and cancer. It has also been implicated in the control of pro-aging signalling pathways, which have

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a significant overlap with tumour growth pathways.<sup>2</sup> AMPK is activated by cellular stress resulting in the restoration of energy levels through regulation of metabolism and growth.<sup>3</sup> Insufficient activity of AMPK allows uncontrolled cell growth, despite the conditions of cellular stress such as those occurring during tumourigenesis, making AMPK an attractive target for anti-cancer therapy. Although numerous AMPK-activating drugs have been described, only one of these, metformin, is widely used clinically in the treatment of diabetes mellitus type 2. Other AMPK activators are currently less suitable for routine clinical use due to the higher rates of lactic acidosis (phenformin) or low specificity and potency (5-aminoimidazole-4-carboxamide ribonucleoside). Novel, more specific AMPK activators are still in preclinical development. A rapidly increasing body of preclinical and clinical evidence demonstrates anti-cancer effects of metformin. Other anti-diabetic drugs, such as thiazolidinediones and glucagon-like peptide-1 analogues, may have similar effects but are less widely used and consequently less data are available for these drugs.

This review will discuss how the metabolic disturbances associated with diabetes mellitus type 2 can contribute to carcinogenesis and how the anti-diabetic drug metformin may reverse these disturbances and inhibit cancer growth, both in diabetic and in non-diabetic patients.

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## 2. Diabetes mellitus type 2

Insulin is essential for glucose homeostasis and is required for glucose uptake into cells and conversion of glucose to glycogen for storage. In addition, insulin has anabolic effects including inhibition of gluconeogenesis and cell growth stimulation. Diabetes mellitus is characterised by hyperglycaemia due to an absolute or relative insulin deficiency. Diabetes mellitus type 1, accounting for 5–10% of diabetes patients, is the result of an absolute insulin deficiency due to autoimmune destruction of insulin-secreting pancreatic  $\beta$ -cells. The more prevalent diabetes mellitus type 2 is characterised by a relative insulin deficiency due to reduced tissue responsiveness to insulin. This is known as insulin resistance and it results in insufficient peripheral glucose elimination, impaired inhibition of hepatic gluconeogenesis and, consequently, increased glucose levels. Compensatory insulin secretion increases until  $\beta$ -cell function becomes insufficient due to exhaustion or destruction of the pancreatic  $\beta$ -cells resulting in hyperglycaemia. The chronic hyperglycaemia of diabetes results in long-term microvascular complications including retinopathy, nephropathy and neuropathy. In addition, there is an increased risk of cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension, obesity and abnormalities of lipoprotein metabolism frequently coexist in patients with diabetes mellitus type 2 further increasing these risks. In Europe, over 8% of the general population has diabetes mellitus type 2, and incidence and prevalence are rising rapidly.<sup>4</sup>

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## 3. Diabetes mellitus type 2 and cancer risk

Both diabetes mellitus type 2 and cancer are diseases of the elderly and through chance alone many patients will have

both diagnoses. Besides, numerous conditions associated with hyperinsulinaemia and type 2 diabetes, including physical inactivity, obesity and a high-saturated-fat diet, are independent risk factors for cancer (reviewed in [4]). There is a large overlap in causes and consequences of these conditions, however, there is evidence from case-control and prospective cohort studies that diabetes mellitus type 2 is an independent risk factor for cancer development. Although these studies have inherent methodological problems, meta-analyses show consistent results. Table 1 shows the pooled relative risks of case-control and prospective cohort studies in various cancer types. Overestimation of the risk due to insufficient correction for other risk factors is a danger, as well as publication bias for positive studies, although the funnel plots did not indicate this. Underestimation of the risk is another danger since the control groups may have included undiagnosed diabetics and a proportion of study groups included type 1 diabetics, the majority of which are insulin sensitive and not thought to have an increased cancer risk.<sup>6</sup> Most studies are corrected for these factors to some extent and in general these studies indicate a relatively consistent association between diabetes mellitus type 2 and increased cancer risk.

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## 4. Diabetes mellitus type 2 and cancer outcome

The prevalence of diabetes mellitus type 2 in newly diagnosed cancer patients is estimated to be 8–18%.<sup>7</sup> Epidemiological studies have shown that diabetic patients with cancer may have worse outcomes than their non-diabetic counterparts. A meta-analysis of these studies reported a pooled hazard ratio for the risk of long-term, all-cause mortality of 1.41 [95% confidence interval (CI) 1.28–1.55] in diabetic patients with cancer as compared to non-diabetic patients with cancer.<sup>8</sup> The evidence for increased cancer-site specific mortality risk reached statistical significance for endometrial, breast and colorectal cancer. There are, however, numerous problems interpreting the results of the heterogeneous studies included in this meta-analysis. Clinical treatment decisions in these patients may be influenced by co-morbid conditions, such as ischaemic heart disease, leading to less aggressive treatment regimen. Diabetic patients are generally less health conscious than non-diabetic patients resulting in presentation with later stage disease and, in addition, their higher inherent risk of cardiovascular mortality may be amplified due to reduced physician and patient motivation for cardiovascular risk management during and after cancer treatment. More robust evidence is available from a retrospective analysis of the clinical trial data. Lower pathological complete response (pCR) rates were reported in diabetic patients as compared to non-diabetic patients receiving equivalent neoadjuvant chemotherapy for breast cancer, indicating that these patients may be less susceptible to chemotherapy.<sup>9</sup> Unfortunately, due to the retrospective character of the study, no data were available on insulin levels, degree of insulin resistance or glycosylated haemoglobin levels as a measure of diabetic control. Therefore, although this result indicates that increased insulin levels or insulin resistance may influence

**Table 1 – Meta-analyses: diabetes as a risk factor for cancer.**

Author	Tumour type	Case-control studies		Prospective cohort studies	
		#	RR (95% CI)	#	RR (95% CI)
Larsson et al. <sup>78</sup>	Bladder	7	1.4 (1.0–1.8)	3	1.4 (1.2–1.7)
Larsson et al. <sup>79</sup>	Breast	5	1.2 (1.1–1.3)	15	1.2 (1.1–1.3)
Wolf et al. <sup>80</sup>	Breast	4	1.1 (1.0–1.3)	6	1.3 (1.2–1.3)
Larsson et al. <sup>81</sup>	Colorectal	6	1.4 (1.2–1.5)	9	1.3 (1.2–1.4)
Friberg et al. <sup>82</sup>	Endometrium	13	2.2 (1.8–2.7)	3	1.6 (1.2–2.2)
El-Serag et al. <sup>83</sup>	HCC	13	2.5 (1.9–3.2)	12	2.5 (1.9–3.2)
Chao et al. <sup>84</sup>	NHL	10	1.2 (1.0–1.4)	3	1.8 (1.3–2.5)
Mitri et al. <sup>85</sup>	NHL	11	1.1 (0.9–1.3)	5	1.4 (1.1–1.9)
Everhart et al. <sup>86</sup>	Pancreatic	11	1.8 (1.1–2.7)	9	2.6 (1.6–4.1)
Huxley et al. <sup>87</sup>	Pancreatic	17	1.9 (1.5–2.5)	19	1.7 (1.6–1.9)
Bonovas et al. <sup>88</sup>	Prostate	5	0.9 (0.7–1.2)	9	0.9 (0.9–1.0)
Kasper et al. <sup>89</sup>	Prostate	7	0.9 (0.7–1.1)	12	0.8 (0.7–0.9)

RR: pooled relative risk. CI: confidence interval. HCC: hepatocellular carcinoma. NHL: non-Hodgkin's lymphoma. #Number of studies.

efficacy of anti-cancer agents, it requires confirmation in more robust prospective studies.

## 5. Diabetes and cancer: mechanisms

The pathophysiological mechanisms responsible for the development of diabetes mellitus type 2, insulin resistance, hyperglycaemia and the resulting hyperinsulinaemia are all associated with cancer risk.<sup>5</sup> Although insulin responsive tissues, such as skeletal muscle, become insulin resistant, the epithelial cells remain relatively insulin sensitive and increased insulin-mediated signalling can lead to enhanced proliferation, as has been demonstrated in both cell line models and rodent studies.<sup>10</sup> Furthermore, there is evidence from animal models that hyperinsulinaemia in the setting of insulin resistance as well as exogenous insulin injections increases susceptibility to chemically induced carcinogenesis.<sup>11,12</sup> In addition, the use of exogenous insulin and possibly insulin secretagogues, such as sulfonylurea drugs, in the treatment of patients with diabetes mellitus type 2 has been associated with an increased risk of cancer and cancer recurrence.<sup>13</sup> Insulin resistance and the subsequent hyperinsulinaemia may also explain the increased cancer risk in other conditions such as obesity, high fat diets and the metabolic syndrome. Life-style interventions, such as weight loss and exercise, reduce insulin resistance in diabetic patients and also lower long-term mortality in cancer patients.<sup>14</sup> Insulin and hyperinsulinaemia can promote tumorigenesis directly through the insulin receptor in epithelial tissues or indirectly by affecting the levels of other modulators, such as insulin-like growth factors (IGFs), sex hormones, inflammatory processes and adipokines (Fig. 1).

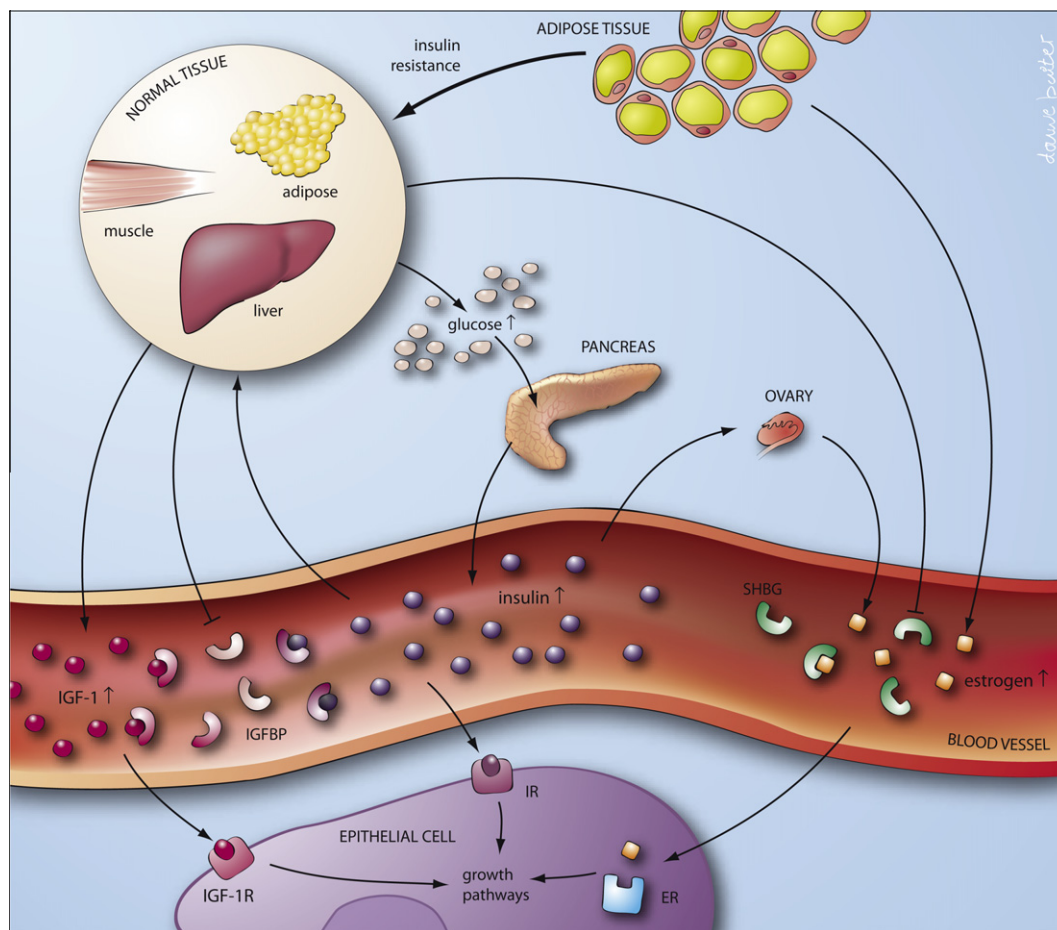
### 5.1. Insulin receptor signalling

In humans, insulin receptors are widely expressed both in normal tissues and in primary cancers.<sup>15</sup> In contrast to normal tissues which commonly express the insulin receptor B isoform, cancer cells preferentially express the insulin receptor A isoform which may differ from the B isoform in its affinity for the various ligands.<sup>10</sup> In addition, cancer cells appear to

have lost their ability to down-regulate insulin receptors in response to hyperinsulinaemia potentially explaining the influence of insulin levels on cancer prognosis.<sup>16</sup> Insulin binds to preassembled receptor heterodimers and initiates signalling through adaptor proteins, the insulin receptor substrates (IRS1-4), activating the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein-kinase (MAPK) signalling pathways and resulting in a cascade of proliferative and anti-apoptotic events.<sup>17</sup> The PI3K pathway mediates the glucose regulatory effects of insulin but is inhibited in insulin resistance and, therefore, hyperinsulinaemia, leading to increased signal transduction, is required to restore normal PI3K pathway activity. Since signalling via the MAPK pathway is preserved despite insulin resistance, this results in hyperactivation of this pathway and enhanced cellular proliferation in the setting of hyperinsulinaemia.<sup>5</sup>

### 5.2. Insulin-like growth factor signalling

The insulin and IGF-1 receptors (IGF-1Rs) are highly homologous and both insulin and IGFs can bind to and activate the IGF-1R which is frequently overexpressed in cancer cells.<sup>18</sup> Functional insulin receptor-IGF-1R heterodimers also occur. Epidemiological evidence supports a role for elevated circulating IGF-1 levels in the development of a variety of cancers, including colorectal, prostate and breast cancers.<sup>19</sup> Hyperinsulinaemia can result in high IGF-1 levels through various mechanisms. Insulin can upregulate IGF-I by displacing it from common binding proteins and can stimulate IGF-1 release by the upregulation of hepatic growth hormone signalling during hyperinsulinaemia.<sup>20</sup> A similar mechanism can lead to increased IGF-II levels.<sup>21</sup> In addition, hyperinsulinaemia suppresses the levels of IGF-binding proteins leading to increased availability of bioactive IGF-1.<sup>22</sup> High IGF-1 levels promote cancer cell growth, both *in vitro* and *in vivo*, by signalling through the IGF-1R.<sup>23</sup> This leads to PI3K and MAPK pathway activation and mitogenic effects that appear stronger than for insulin receptor-mediated signalling.<sup>24</sup> Little is currently known regarding the roles of IGF-II, the different insulin receptor isoforms and insulin receptor/IGF-1R heterodimers in carcinogenesis in patients with diabetes mellitus type 2.



**Fig. 1 – Mechanisms resulting in tumour growth in patients with diabetes mellitus type 2.** IR: insulin receptor. IGF-I: insulin-like growth factor-I. IGF-1R: insulin-like growth factor receptor 1. ER: oestrogen receptor. SHBG: sex-hormone-binding globulin. IGFBP: IGF-binding protein. A combination of genetic predisposition, inflammation and obesity can cause insulin resistance. This leads to increased glucose levels causing increased insulin production by the pancreas until eventually the pancreatic  $\beta$ -cells become exhausted. Increased insulin levels lead to increased binding to IGFBPs, displacing IGFs from these binding proteins and resulting in increased free IGF-1 levels. Insulin resistance also inhibits IGFBP and increases IGF-1 production in the liver both contributing to increase in free IGF-1 levels. High insulin levels increase oestrogen production in the ovary, adipose tissue increases the conversion of androgens to oestrogens and insulin resistance inhibits the production of sex-hormone-binding globulin all leading to increased levels of free oestrogens. High levels of insulin, IGF-1 and oestrogen can all stimulate growth pathways in epithelial cells through their respective receptors promoting carcinogenesis or cancer progression.

### 5.3. Sex hormones

The existence of a relationship between insulin resistance and sex hormone levels is illustrated in polycystic ovary syndrome, the most common cause of anovulatory infertility, characterised by anovulation, hyperandrogenaemia and insulin resistance. Insulin resistance and hyperinsulinaemia suppress the production of sex hormone-binding globulin by the liver.<sup>25</sup> This can lead to increased availability of free sex hormones favouring the development of sex hormone-dependent cancers such as breast cancer.<sup>26</sup> Increased conversion of androgens to oestrogens in adipose tissue can also increase free oestrogen levels adding to this effect. Negative feedback through the hypothalamus and pituitary gland, which should correct these increased levels, appears to be disrupted in diabetic patients as evidenced by increased levels of luteinising

hormone and follicle stimulating hormone.<sup>27</sup> Interestingly, low levels of sex hormone-binding globulins and high levels of oestradiol also predict the development of diabetes mellitus type 2, possibly through their association with early insulin resistance.<sup>28</sup>

### 5.4. Inflammation and adipokines

Inflammation provokes insulin resistance and pancreatic  $\beta$ -cell dysfunction.<sup>29</sup> Insulin and hyperglycaemia mediate the inflammatory response and inflammatory markers including C-reactive protein and tissue plasminogen activator are independent predictors of the development of type 2 diabetes.<sup>30</sup> In adiposity, non-esterified fatty acids compete with glucose as a metabolic fuel, inducing insulin resistance, increasing fatty acid oxidation and resulting in reactive oxygen species



(ROS) production. Macrophages in adipose tissue can release cytokines and other signalling proteins (known as adipokines), including leptin, adiponectin, tumour necrosis factor- $\alpha$  and interleukin-6.<sup>31</sup> The majority of these inflammatory mediators are known to increase insulin resistance leading to increases in insulin levels which contribute to an enhanced inflammatory response.<sup>29</sup> Chronic, sub-clinical inflammation is believed to be a key factor in tumourigenesis and cancer progression.<sup>32</sup>

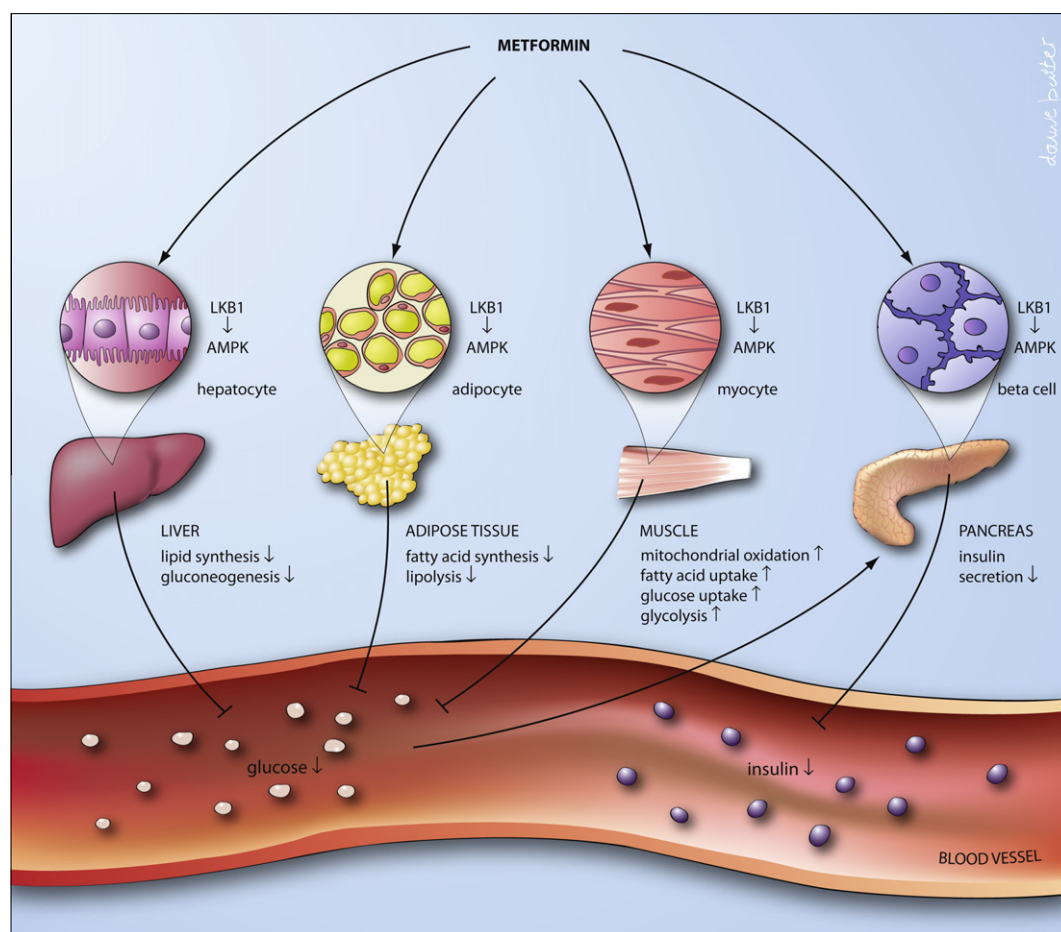
## 6. Metformin in diabetes: clinical use

Metformin, a biguanide derivative, is a widely prescribed oral drug used as first-line therapy for diabetes mellitus type 2. The primary actions of metformin are inhibition of hepatic glucose production and reduction of insulin resistance in peripheral tissue leading to enhanced glucose uptake and utilisation in skeletal muscle. This reduces the levels of circulating glucose and decreases the plasma insulin levels improving long-term glycaemic control and reducing the incidence of diabetes-related complications. Metformin is an inexpensive and safe drug, with minor gastrointestinal upset being the most common toxicity. The most serious toxicity is

lactic acidosis, occurring in 3/100,000 patients years of use, the risk of which is significantly reduced if metformin is avoided in patients with hepatic, cardiac or renal compromise and in patients older than 80 years.

## 7. Metformin in diabetes: mechanisms of action

The principal mediator of the glucose- and insulin-lowering effects of metformin is AMPK activation, through activation of the upstream kinase liver-kinase B1 (LKB1), resulting in the inhibition of gluconeogenesis.<sup>33</sup> AMPK is a central cellular energy sensor which responds to increases in the adenosine monophosphate/adenosine triphosphate ratio.<sup>34</sup> Physiological conditions of nutrient deprivation activate AMPK leading to inhibition of energy-consuming processes and stimulation of processes that generate energy, resulting in restoration of the adenosine triphosphate supply (Fig. 2).<sup>3</sup> One of the major growth regulatory pathways controlled by AMPK is the mammalian target of rapamycin (mTOR) pathway and its downstream substrates, such as the ribosomal S6 kinase (S6K1).<sup>35</sup> This pathway regulates protein translation of cell growth regulators such as cyclin D1, hypoxia inducible factor 1 $\alpha$  (HIF1 $\alpha$ )



**Fig. 2 – Effects of metformin in normal tissues in patients with diabetes mellitus type 2.** LKB1: liver kinase B1. AMPK: AMP-activated protein kinase. Metformin activates LKB1 which in turn activates AMPK leading to differential effects in various tissues. The net result of these effects is down-regulation of processes that consume energy and up-regulation of processes that generate energy resulting in reductions in serum glucose and insulin levels.

and MYC which control processes such as cell cycle progression, cell growth and angiogenesis.<sup>36</sup> In patients with diabetes mellitus type 2 activation of AMPK by metformin results in partial reversal of metabolic disturbances such as hyperglycaemia and insulin resistance.

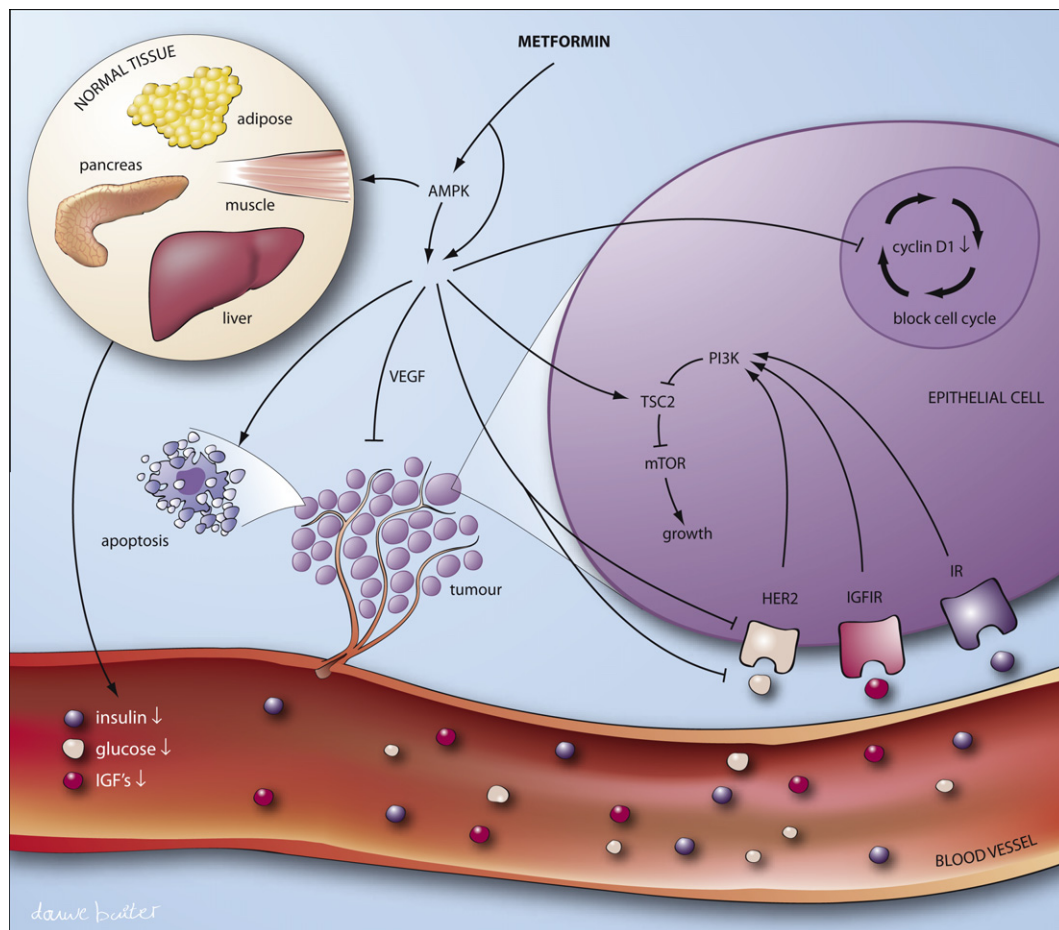
## 8. Metformin as an anti-cancer agent: potential mechanisms of action

The elucidation of the role of AMPK in metabolism in combination with increasing evidence linking the metabolic abnormalities associated with diabetes mellitus type 2 to cancer has generated profound interest in metformin as an anti-cancer agent. The beneficial effects expected from the reversal of hyperglycaemia, insulin resistance and hyperinsulinaemia

and their mitogenic effects have indeed been demonstrated in *in vitro* and *in vivo* models of cancer. Reported mechanisms of action for metformin include reduced insulin-like growth factor, insulin and HER2-mediated signalling, inhibition of mTOR signalling, inhibition of angiogenesis and induction of cell cycle arrest and apoptosis (Fig. 3).

### 8.1. Cell growth inhibition

Metformin inhibits the growth of various types of cancer cells both *in vitro* and *in vivo*.<sup>37</sup> This inhibition is both dose- and time dependent and can potentiate the effect of chemotherapy.<sup>38,39</sup> Growth inhibition is (partially) abolished in the presence of small interfering RNAs against AMPK or AMPK inhibitors demonstrating the pivotal role of AMPK.<sup>40</sup> LKB1



**Fig. 3 – Anti-cancer effects of metformin.** IGF-I: insulin-like growth factor 1. IGF-1R: insulin-like growth factor 1. IR: insulin receptor. VEGF: vascular endothelial growth factor. AMPK: AMP activated protein kinase. HER2: epithelial growth factor receptor 2. PI3K: phosphoinositide 3-kinase. TSC2: tuberous sclerosis complex 2. mTOR: mammalian target of rapamycin. Metformin activates AMPK in liver, muscle, adipose tissue and pancreas resulting in reduced levels of insulin and IGF-I. This, in turn, leads to reduced growth pathway signalling through their respective receptors. Through AMPK-dependent or -independent mechanisms, metformin can lead to several other anti-tumour effects. Firstly, metformin can inhibit mTOR signalling through phosphorylation and stabilisation of TSC2. Secondly, metformin can suppress HER-2 protein expression and also inhibit HER-2 protein kinase activation resulting in reduced signalling through downstream pathways. Thirdly, metformin can decrease levels of VEGF resulting in inhibition of angiogenesis. Fourthly, metformin can induce apoptosis through p53-dependent or -independent mechanisms. Lastly, metformin can block cell cycle arrest at least partially mediated through reduced cyclin D1 expression.

expression is essential for the activation of AMPK by metformin. Metformin does not inhibit cell growth in LBK1 null cells confirming the requirement of functional LBK1 for metformin induced AMPK activation.<sup>40</sup>

### 8.2. Insulin-like growth factor signalling

Metformin reverses hyperinsulinaemia through its effects on glucose homeostasis, both in diabetes mellitus type 2 patients and in non-diabetic hyperinsulinaemic patients, and may have anti-proliferative effects via this mechanism.<sup>41</sup> Besides lowering insulin levels, metformin can indirectly lower IGF-I levels through effects on levels of insulin and insulin-binding proteins (Fig. 3).<sup>10</sup> Furthermore, metformin can decrease IGF-mediated signalling by inhibiting tyrosine kinase phosphorylation of the adaptor protein IRS-1 and interrupt crosstalk between insulin/IGF-1 receptors and G protein-coupled receptor signalling systems.<sup>42,43</sup>

### 8.3. mTOR pathway inhibition

The majority of the growth inhibitory effects of metformin are mediated through the inhibition of mTOR signalling (Fig. 3).<sup>40</sup> Activation of AMPK by metformin results in phosphorylation and stabilisation of tuberous sclerosis complex, which integrates regulatory inputs and transmits them to mTOR. These regulatory inputs include oxygen-dependent signals and growth factor-dependent signalling pathways such as the PI3K and the MAPK signalling pathways.<sup>44</sup> mTOR phosphorylates down-stream mediators leading to the regulation of cell cycle progression, cell growth and angiogenesis. mTOR signalling is increased in most common human cancers and activation of mTOR-dependent protein translation correlates with malignant progression, adverse prognosis and resistance to both chemotherapy and targeted therapy such as trastuzumab.<sup>45</sup> Clinical trials using rapamycin analogues, such as temsirolimus and everolimus, currently registered for the treatment of advanced renal cell cancer, have validated the importance of mTOR inhibition as an anti-cancer treatment strategy. However, the anti-tumour activity as a single agent in renal cell cancer is modest. Interestingly, preclinical studies indicate that metformin inhibits cell survival to a greater extent than the mTOR inhibitor rapamycin.<sup>46</sup>

### 8.4. HER-2 expression and signalling

HER2 is overexpressed in approximately 20% of breast cancers and is a major driver of proliferation in cancer cells. Effects of metformin on HER-2 expression and signalling have been described (Fig. 3). Metformin reduces HER-2 protein expression in human breast cancer cells through inhibition of mTOR.<sup>47</sup> Interestingly, at lower concentrations, metformin blocks HER-2 kinase activity. Metformin can prevent resistance to HER-2-based therapies by inhibiting the upregulation of survivin.<sup>48</sup> In a similar way, mTOR inhibitors can overcome trastuzumab resistance. Interestingly, AMPK activation allows cardiac cells to survive the cardiotoxic effects of anti-HER-2 therapy.<sup>49</sup> These mechanisms indicate that combinational therapy with HER-2-targeted agents and metformin could have synergistic effects.

### 8.5. Angiogenesis and inflammation

Inhibition of angiogenesis is another proposed mechanism of metformin's effect (Fig. 3). Metformin attenuates angiogenic stimuli in the serum of polycystic ovarian syndrome patients with insulin resistance and decreases levels of vascular endothelial growth factor (VEGF) in obese diabetic patients.<sup>50</sup> In addition, *in vitro* studies have shown inhibition of angiogenesis and inflammation by metformin through inhibition of mediators such as HIF-1 $\alpha$ , tumour necrosis factor alpha, plasminogen activator inhibitor-1 antigen and von Willebrand factor, possibly through inhibition of mTOR signalling.<sup>51</sup> Surprisingly, metformin induced an angiogenic phenotype in MB-435 breast cancer cells leading to enhanced tumour progression in a nude mouse model.<sup>52</sup> This report has been criticised because of the unusual properties of the cell line used and the fact that the dose of metformin used was 40–50 times higher than the recommended human dose.<sup>53</sup> Therefore, although metformin is expected to inhibit angiogenesis, further studies are required to exclude significant pro-angiogenic effects.

### 8.6. Apoptosis and p53

p53 is a tumour suppressor gene involved in DNA-damage repair and cell cycle regulation. Interestingly, in adipose tissue, p53 is involved in the development of insulin resistance through induction of senescence.<sup>54</sup> AMPK can phosphorylate and activate the tumour suppressor p53 leading to the inhibition of cell division and induction of apoptosis in cells that encounter low nutrient conditions.<sup>55</sup> This mechanism can lead to apoptosis in p53 proficient cells and induce re-expression of functional p53 in cells with low levels of wild-type p53.<sup>56</sup> Interestingly, activation of AMPK by metformin also enhances apoptosis induction in p53-deficient colon cancer cell lines, possibly due to a metabolic conversion that p53-deficient cells are unable to execute, making metformin selectively toxic to these cells.<sup>57</sup> Apoptosis induction by metformin is an interesting mechanism although the conditions under which it occurs need to be determined since not all studies describe apoptosis induction by metformin. p53 expression in adipose tissue is involved in the development of insulin resistance and therefore metformin-induced p53 expression may be expected to increase insulin resistance. Non-apoptotic effects of metformin on p53-induced insulin resistance have, however, not been described and if present are likely to be masked by larger reductions in insulin resistance due to improved signalling through the insulin receptor.

### 8.7. Cell cycle arrest

Induction of cell cycle arrest is another potential mechanism of metformin's anti-cancer effect (Fig. 3). In cancer cell lines, metformin treatment resulted in the dose-dependent inhibition of proliferation through a decrease in cyclin D1 protein expression.<sup>38</sup> Genome wide analyses have also demonstrated that metformin suppresses numerous mitosis-related gene families including tubulins, histones and aurora kinases.<sup>58</sup> Further studies are needed to determine the importance of this mechanism in a variety of human tumours since metfor-



min-induced cell cycle arrest requires the presence of cyclin dependent kinase inhibitors which are lost or down-regulated in many cancers.

#### 8.8. Vitamin B12 deficiency

Long-term use of metformin has been shown to cause vitamin B12 malabsorption.<sup>59</sup> It has been suggested that this may augment the anti-tumour effect of metformin, since a deficiency of metabolically active vitamin B12 can increase tissue toxicity of adjuvant chemotherapy.<sup>60</sup> This potential mechanism deserves further attention in the clinical situation, especially since vitamin B12 deficiency can also have detrimental effects including neuropathy and anaemia.

### 9. Metformin in cancer: preclinical studies

Studies in rodent models confirmed that metformin induces AMPK activation, can inhibit tumour growth and prevent or delay tumour development. Metformin significantly reduced the stimulatory effect of a high energy diet on the growth of lung cancer xenografts in mice as compared to placebo.<sup>61</sup> In carcinogen-induced rodent models of colon and mammary cancer metformin delayed tumour growth.<sup>62</sup> Interestingly, metformin also increased the lifespan of mice carrying the HER-2 oncogene by decreasing the size and incidence of spontaneous mammary tumours.<sup>63</sup> These, limited, preclinical studies support the further development of metformin as an anti-cancer treatment but further studies are essential to clarify the most promising settings, identify potentially synergistic effects with other anti-tumour agents and predict adverse effects.

### 10. Metformin in cancer: population studies

Three population-based studies have suggested that metformin decreases the incidence of cancer and cancer-related mortality in diabetic patients.<sup>13,64,65</sup> Evans et al. showed that the risk of cancer was reduced in patients with diabetes mellitus type 2 receiving metformin (odds ratio 0.85 for any metformin exposure versus no metformin exposure).<sup>64</sup> In the same population, new metformin users were at a lower risk of cancer than the matched controls (adjusted hazard ratio 0.53–0.75).<sup>66</sup> Landman et al. showed that metformin use was associated with lower cancer mortality compared to no metformin use (hazard ratio 0.23–0.80) and that the effect was dose dependent.<sup>65</sup> Bowker et al. showed that cancer-related mortality was lower in patients with diabetes receiving metformin compared to patients receiving sulfonylureas or insulin (hazard ratio 0.55–0.77).<sup>13</sup> Unfortunately, there was no untreated control group so it is not possible to determine whether metformin reduced cancer risk or insulin (secretalogues) increased cancer risk. Recent meta-analyses, however, do not indicate a carcinogenic effect of insulin (secretalogues) suggesting a protective effect of metformin.<sup>67</sup> Although many confounding factors complicate the interpretation of these studies, including the severity of the diabetes and other reasons determining metformin use, they suggest an anti-cancer effect.

### 11. Metformin in cancer: retrospective clinical study

Jiralersprong et al. studied chemotherapy response rates in a group of 2592 patients treated with neoadjuvant chemotherapy for early stage or locally advanced breast cancer. They found that in 157 diabetic patients, metformin use was an independent predictor of pathological complete response (pCR) with 24% of metformin users achieving pCR compared to 8% in diabetic patients not using metformin.<sup>9</sup> Insulin use decreased the pCR rate in diabetic patients not using metformin, while there was no influence of insulin in metformin users. This suggests that metformin reverses the negative effects of insulin use on the pCR rate in diabetic patients using insulin, while increasing the pCR rate in diabetic patients not using insulin.<sup>9</sup> Unfortunately, in this study, no information is available on glycosylated haemoglobin levels or other parameters of diabetic control and it is possible that improved diabetic control due to metformin rather than metformin itself increased the pCR rate. Although metformin treatment did not influence overall survival in this small retrospective study, pCR is a recognised surrogate end-point for survival in neo-adjuvant studies and these impressive results have lead to a huge interest in metformin as an anti-cancer agent.

### 12. Metformin in cancer: planned trials and trial design

The data described in the previous sections strongly support the clinical development of metformin as an anti-cancer agent. There are currently numerous ongoing prospective clinical studies investigating the safety and/or efficacy of metformin in patients with cancer as described in Table 2. Only two of these trials include determination of the maximum tolerated dose as an end-point. Although there is an extensive clinical experience with metformin in patients with insulin resistance, care is required when giving this drug to insulin-sensitive patients and attention to the safety profile of metformin in these patients will be essential in early trials. The retrospective data in neo-adjuvant breast cancer therapy indicate that metformin may potentiate traditional chemotherapeutic agents. *In vitro* studies indicate that the anti-tumour effect of metformin is at least as strong as for mTOR inhibitors. Combinational therapy with other drugs inhibiting the mTOR pathway is especially interesting since metformin can also counteract the increased glucose levels resulting from mTOR inhibition. Combination with HER2 inhibitors also has good potential based on *in vitro* data showing that metformin can overcome trastuzumab resistance and may protect cardiac cells from HER2-inhibition-related cardiotoxicity.

The development of predictive serum and tissue biomarkers of metformin effect is essential for the clinical development of metformin as an anti-cancer agent. Candidates based on preclinical data include p53 status and markers of mTOR activation such as S6K phosphorylation. It will also be important to determine whether some degree of insulin resistance is required for the anti-tumour effects of metformin and the role played by altered IGF signalling. Other



**Table 2 – Registered and announced prospective trials of metformin in cancer.**

Reference	Tumour type	Design	Setting	Concomitant treatment	Primary outcome
NCT00659568	Solid tumours	Phase 1b	Advanced	Temsirolimus	MTD
NCT01087983	Solid tumours	Phase 1	Advanced	Lapatinib	MTD
NCT00984490	Breast	Phase I	Neoadjuvant		Proliferation apoptosis
NCT00930579	Breast	Phase I	Neoadjuvant		AMPK mTOR
NCT00897884	Breast	Phase II	Neoadjuvant		Proliferation
NCT00881725	Prostate	Phase II	Neoadjuvant		pAKT
Martin-Castillo et al. <sup>90</sup>	Breast, HER-2+	Phase II	Neoadjuvant		Unknown
NCT00909506	Breast	Phase II <sup>a</sup>	Adjuvant	Tamoxifen	Weight loss
Muti et al. <sup>91</sup>	Breast	Phase II <sup>a</sup>	Prevention		Breast cancer
Cazzaniga et al. <sup>92</sup>	Breast	Phase II <sup>a</sup>	Neoadjuvant		Proliferation
NCT01101438	Breast	Phase III	Adjuvant	Standard	Survival

MTD: maximum tolerated dose; pAKT: phosphorylated AKT; AMPK: adenosine monophosphate kinase; mTOR: mammalian target of rapamycin.

<sup>a</sup> Placebo controlled.

potentially predictive factors include OCT1 polymorphisms and LKB1 mutation status. The cell surface transporter cation 1 (OCT1) is essential for efficient metformin uptake in hepatocytes and polymorphisms underlie metformin resistance in patients with diabetes mellitus type 2.<sup>68</sup> OCT1 expression has been demonstrated in cancer cell lines and some human cancers but it is currently unclear whether OCT1 is widely expressed in human cancers.<sup>69</sup> Expression of the tumour suppressor gene LKB1 is essential for AMPK activation by metformin, and in polycystic ovarian syndrome patients LKB1 polymorphisms are found in metformin non-responders.<sup>70</sup> Somatic mutations in LKB1 are observed in up to 30% of sporadic tumours, including pulmonary and colorectal tumours.<sup>71</sup> Exploration of the anti-cancer effects of AMPK activators, such as the AMPK-binding small molecule A769662, which do not require functional LKB1, is another promising strategy. A769662 is a more potent activator of AMPK than metformin and consequently suppressed the mTOR pathway in a greater range of tissues and delayed tumour onset in PTEN-deficient mice more efficiently than metformin.<sup>72</sup>

The value of (metabolic) tumour imaging is another relevant and interesting question. Through AMPK, metformin treatment mediates changes in the tumour metabolism and therefore positron emission tomography (PET) combined with fluorine-18-labelled-fluoro-deoxy-glucose ([<sup>18</sup>F]FDG) imaging may be used to determine tumour response to metformin. FDG-PET imaging studies do not describe changed normal tissue uptake of glucose in patients treated with metformin, although increased hepatic glucose uptake and colonic uptake have been demonstrated.<sup>73–76</sup> Preclinical studies are required to determine whether FDG-PET imaging will be suitable for measuring tumour response to metformin. An alternative may be the use of stably labelled glucose which can be used to obtain detailed information on glucose kinetics without exposing the patient to ionising radiation.<sup>77</sup>

### 13. Conclusion

In conclusion, there is a large and rapidly expanding body of evidence from *in vitro* and *in vivo* models of carcinogenesis supporting the potential efficacy of metformin as an anti-cancer agent and this is supported by population-based stud-

ies and retrospective analyses of clinical studies. It is important that, despite the previous clinical experience with metformin, early clinical trials are well designed, incorporating both safety end-points and translational research to identify appropriate biomarkers. The complex interactions between tumour metabolism and growth are rapidly being elucidated and metformin may prove to be a non-toxic, inexpensive drug which can modulate these tumour stimulatory pathways.

### Conflict of interest statement

None declared.

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### REFERENCES

- Warburg O. On the origin of cancer cells. *Science* 1956;**123**:309–14.
- Vijg J, Campisi J. Puzzles, promises and a cure for ageing. *Nature* 2008;**454**:1065–71.
- Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. *Circ Res* 2007;**100**:328–41.
- King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. *Diabetes Care* 1998;**21**:1414–31.
- Godsland IF. Insulin resistance and hyperinsulinaemia in the development and progression of cancer. *Clin Sci (Lond)* 2009;**118**:315–32.
- Hjalgrim H, Frisch M, Ekblom A, et al. Cancer and diabetes. A follow-up study of two population-based cohorts of diabetic patients. *J Intern Med* 1997;**241**:471–5.
- Goodwin PJ, Ennis M, Pritchard KI, et al. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. *J Clin Oncol* 2002;**20**:42–51.
- Barone BB, Yeh HC, Snyder CF, et al. Long-term all-cause mortality in cancer patients with preexisting diabetes

- mellitus: a systematic review and meta-analysis. *JAMA* 2008;**300**:2754–64.
9. Jiralerspong S, Palla SL, Giordano SH, et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J Clin Oncol* 2009;**27**:3297–302.
  10. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 2008;**8**:915–28.
  11. Koohestani N, Tran TT, Lee W, Wolever TM, Bruce WR. Insulin resistance and promotion of aberrant crypt foci in the colons of rats on a high-fat diet. *Nutr Cancer* 1997;**29**:69–76.
  12. Tran TT, Medline A, Bruce WR. Insulin promotion of colon tumors in rats. *Cancer Epidemiol Biomarkers Prev* 1996;**5**:1013–5.
  13. Bowker SL, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care* 2006;**29**:254–8.
  14. Chlebowski RB, Hoy MK, Thomson CA, et al. Survival analyses from the Women's Intervention Nutrition Study (WINS) evaluating dietary fat reduction and breast cancer outcome. *JCO* 2008;**26**:522.
  15. Frasca F, Pandini G, Sciacca L, et al. The role of insulin receptors and IGF-I receptors in cancer and other diseases. *Arch Physiol Biochem* 2008;**114**:23–37.
  16. Mountjoy KG, Finlay GJ, Holdaway IM. Abnormal insulin-receptor down regulation and dissociation of down regulation from insulin biological action in cultured human tumor cells. *Cancer Res* 1987;**47**:6500–4.
  17. Papa V, Belfiore A. Insulin receptors in breast cancer: biological and clinical role. *J Endocrinol Invest* 1996;**19**:324–33.
  18. De Meyts P. Insulin and its receptor: structure, function and evolution. *Bioessays* 2004;**26**:1351–62.
  19. Ibrahim YH, Yee D. Insulin-like growth factor-I and cancer risk. *Growth Horm IGF Res* 2004;**14**:261–9.
  20. Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK. Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. *J Clin Endocrinol Metab* 2000;**85**:4712–20.
  21. Clemmons DR, Slevi M, Allan G, Sommer A. Effects of combined recombinant insulin-like growth factor (IGF)-I and IGF binding protein-3 in type 2 diabetic patients on glycemic control and distribution of IGF-I and IGF-II among serum binding protein complexes. *J Clin Endocrinol Metab* 2007;**92**:2652–8.
  22. Snyder DK, Clemmons DR. Insulin-dependent regulation of insulin-like growth factor-binding protein-1. *J Clin Endocrinol Metab* 1990;**71**:1632–6.
  23. Wu Y, Yakar S, Zhao L, Hennighausen L, LeRoith D. Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis. *Cancer Res* 2002;**62**:1030–5.
  24. Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. *J Natl Cancer Inst* 2000;**92**:1472–89.
  25. Plymate SR, Matej LA, Jones RE, Friedl KE. Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. *J Clin Endocrinol Metab* 1988;**67**:460–4.
  26. Pugeat M, Crave JC, Elmidani M, et al. Pathophysiology of sex hormone binding globulin (SHBG): relation to insulin. *J Steroid Biochem Mol Biol* 1991;**40**:841–9.
  27. Tok EC, Ertunc D, Evruke C, Dilek S. The androgenic profile of women with non-insulin-dependent diabetes mellitus. *J Reprod Med* 2004;**49**:746–52.
  28. Vikan T, Schirmer H, Njolstad I, Svartberg J. Low testosterone and sex hormone-binding globulin levels and high estradiol levels are independent predictors of type 2 diabetes in men. *Eur J Endocrinol* 2010;**162**:747–54.
  29. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006;**116**:1793–801.
  30. Festa A, D'Agostino Jr R, Tracy RP, Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 2002;**51**:1131–7.
  31. Housa D, Housova J, Vernerova Z, Haluzik M. Adipocytokines and cancer. *Physiol Res* 2006;**55**:233–44.
  32. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008;**454**:436–44.
  33. Shaw RJ, Lamia KA, Vasquez D, et al. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* 2005;**310**:1642–6.
  34. Stapleton D, Mitchelhill KI, Gao G, et al. Mammalian AMP-activated protein kinase subfamily. *J Biol Chem* 1996;**271**:611–4.
  35. Shaw RJ, Bardeesy N, Manning BD, et al. The LKB1 tumor suppressor negatively regulates mTOR signaling. *Cancer Cell* 2004;**6**:91–9.
  36. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell* 2007;**12**:9–22.
  37. Alimova IN, Liu B, Fan Z, et al. Metformin inhibits breast cancer cell growth, colony formation and induces cell cycle arrest in vitro. *Cell cycle* 2009;**8**:909–15.
  38. Ben Sahra I, Laurent K, Loubat A, et al. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. *Oncogene* 2008;**27**:3576–86.
  39. Gotlieb WH, Saumet J, Beauchamp MC, et al. In vitro metformin anti-neoplastic activity in epithelial ovarian cancer. *Gynecol Oncol* 2008;**110**:246–50.
  40. Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. *Cancer Res* 2006;**66**:10269–73.
  41. Goodwin PJ, Pritchard KI, Ennis M, et al. Insulin-lowering effects of metformin in women with early breast cancer. *Clin Breast Cancer* 2008;**8**:501–5.
  42. Ning J, Clemmons DR. AMP-activated protein kinase inhibits IGF-I signaling and protein synthesis in vascular smooth muscle cells via stimulation of insulin receptor substrate 1 S794 and tuberous sclerosis 2 S1345 phosphorylation. *Mol Endocrinol* 2010;**24**:1218–29.
  43. Rozengurt E, Sinnett-Smith J, Kisfalvi K. Crosstalk between insulin/insulin-like growth factor-1 receptors and G protein-coupled receptor signaling systems: a novel target for the antidiabetic drug metformin in pancreatic cancer. *Clin Cancer Res* 2010;**16**:2505–11.
  44. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003;**115**:577–90.
  45. Morgensztern D, McLeod HL. PI3K/Akt/mTOR pathway as a target for cancer therapy. *Anticancer Drugs* 2005;**16**:797–803.
  46. Zakikhani M, Blouin MJ, Piura E, Pollak MN. Metformin and rapamycin have distinct effects on the AKT pathway and proliferation in breast cancer cells. *Breast Cancer Res Treat* 2010 [Epub ahead of print].
  47. Vazquez-Martin A, Oliveras-Ferraro C, Menendez JA. The antidiabetic drug metformin suppresses HER2 (erbB-2) oncoprotein overexpression via inhibition of the mTOR effector p70S6K1 in human breast carcinoma cells. *Cell Cycle* 2009;**8**:88–96.
  48. Vazquez-Martin A, Oliveras-Ferraro C, del Barco S, Martin-Castillo B, Menendez JA. The antidiabetic drug metformin: a pharmaceutical AMPK activator to overcome breast cancer resistance to HER2 inhibitors while decreasing risk of cardiomyopathy. *Ann Oncol* 2009;**20**:592–5.
  49. Shell SA, Lyass L, Trusk PB, et al. Activation of AMPK is necessary for killing cancer cells and sparing cardiac cells. *Cell Cycle* 2008;**7**:1769–75.

50. Ersoy C, Kiyici S, Budak F, et al. The effect of metformin treatment on VEGF and PAI-1 levels in obese type 2 diabetic patients. *Diabetes Res Clin Pract* 2008;**81**:56–60.
51. Lund SS, Tarnow L, Stehouwer CD, et al. Impact of metformin versus repaglinide on non-glycaemic cardiovascular risk markers related to inflammation and endothelial dysfunction in non-obese patients with type 2 diabetes. *Eur J Endocrinol* 2008;**158**:631–41.
52. Phoenix KN, Vumbaca F, Claffey KP. Therapeutic metformin/AMPK activation promotes the angiogenic phenotype in the ERalpha negative MDA-MB-435 breast cancer model. *Breast Cancer Res Treat* 2009;**113**:101–11.
53. Stambolic V, Woodgett JR, Fantus IG, Pritchard KI, Goodwin PJ. Utility of metformin in breast cancer treatment, is neoangiogenesis a risk factor? *Breast Cancer Res Treat* 2009;**114**:387–9.
54. Minamino T, Orimo M, Shimizu I, et al. A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat Med* 2009;**15**:1082–7.
55. Thoreen CC, Sabatini DM. AMPK and p53 help cells through lean times. *Cell Metab* 2005;**1**:287–8.
56. Ben Sahra I, Laurent K, Giuliano S, et al. Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. *Cancer Res* 2010;**70**:2465–75.
57. Buzzai M, Jones RG, Amaravadi RK, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res* 2007;**67**:6745–52.
58. Oliveras-Ferraros C, Vazquez-Martin A, Menendez JA. Genome-wide inhibitory impact of the AMPK activator metformin on M-phase cell cycle genes in human breast cancer cells. *Cell Cycle* 2009;**8**:1633–6.
59. de Jager J, Kooy A, Leheret P, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. *BMJ* 2010;**340**:c2181.
60. Garcia A, Tisman G. Metformin, B(12), and enhanced breast cancer response to chemotherapy. *J Clin Oncol* 2010;**28**:e19.
61. Algire C, Zakikhani M, Blouin MJ, Shuai JH, Pollak M. Metformin attenuates the stimulatory effect of a high-energy diet on in vivo LLC1 carcinoma growth. *Endocr Relat Cancer* 2008;**15**:833–9.
62. Bojkova B, Orendas P, Garajova M, et al. Metformin in chemically-induced mammary carcinogenesis in rats. *Neoplasia* 2009;**56**:269–74.
63. Anisimov VN, Berstein LM, Egormin PA, et al. Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. *Exp Gerontol* 2005;**40**:685–93.
64. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. *BMJ* 2005;**330**:1304–5.
65. Landman GW, Kleefstra N, van Hateren KJ, et al. Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. *Diabetes Care* 2010;**33**:322–6.
66. Libby G, Donnelly LA, Donnan PT, et al. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* 2009;**32**:1620–5.
67. Hernandez-Diaz S, Adami HO. Diabetes therapy and cancer risk: causal effects and other plausible explanations. *Diabetologia* 2010;**53**:802–8.
68. Shu Y, Sheardown SA, Brown C, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 2007;**117**:1422–31.
69. Hayer-Zillgen M, Bruss M, Bonisch H. Expression and pharmacological profile of the human organic cation transporters hOCT1, hOCT2 and hOCT3. *Br J Pharmacol* 2002;**136**:829–36.
70. Legro RS, Barnhart HX, Schlaff WD, et al. Ovulatory response to treatment of polycystic ovary syndrome is associated with a polymorphism in the STK11 gene. *J Clin Endocrinol Metab* 2008;**93**:792–800.
71. Hezel AF, Bardeesy N. LKB1; linking cell structure and tumor suppression. *Oncogene* 2008;**27**:6908–19.
72. Cool B, Zinker B, Chiou W, et al. Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. *Cell Metab* 2006;**3**:403–16.
73. Izzo P, Hallsten K, Oikonen V, et al. Effects of metformin and rosiglitazone monotherapy on insulin-mediated hepatic glucose uptake and their relation to visceral fat in type 2 diabetes. *Diabetes Care* 2003;**26**:2069–74.
74. Virtanen KA, Hallsten K, Parkkola R, et al. Differential effects of rosiglitazone and metformin on adipose tissue distribution and glucose uptake in type 2 diabetic subjects. *Diabetes* 2003;**52**:283–90.
75. Hallsten K, Virtanen KA, Lonnqvist F, et al. Enhancement of insulin-stimulated myocardial glucose uptake in patients with Type 2 diabetes treated with rosiglitazone. *Diabet Med* 2004;**21**:1280–7.
76. Gontier E, Fourme E, Wartski M, et al. High and typical 18F-FDG bowel uptake in patients treated with metformin. *Eur J Nucl Med Mol Imaging* 2008;**35**:95–9.
77. van Dijk TH, Laskewitz AJ, Boer TS, et al. Computational analysis of carbohydrate metabolism. 2010;**9**:177–94.
78. Larsson SC, Orsini N, Brismar K, Wolk A. Diabetes mellitus and risk of bladder cancer: a meta-analysis. *Diabetologia* 2006;**49**:2819–23.
79. Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: a meta-analysis. *Int J Cancer* 2007;**121**:856–62.
80. Wolf I, Sadetzki S, Catane R, Karasik A, Kaufman B. Diabetes mellitus and breast cancer. *Lancet Oncol* 2005;**6**:103–11.
81. Larsson SC, Orsini N, Wolk A. Diabetes mellitus and risk of colorectal cancer: a meta-analysis. *J Natl Cancer Inst* 2005;**97**:1679–87.
82. Friberg E, Orsini N, Mantzoros CS, Wolk A. Diabetes mellitus and risk of endometrial cancer: a meta-analysis. *Diabetologia* 2007;**50**:1365–74.
83. El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006;**4**:369–80.
84. Chao C, Page JH. Type 2 diabetes mellitus and risk of non-Hodgkin lymphoma: a systematic review and meta-analysis. *Am J Epidemiol* 2008;**168**:471–80.
85. Mitri J, Castillo J, Pittas AG. Diabetes and risk of Non-Hodgkin's lymphoma: a meta-analysis of observational studies. *Diabetes Care* 2008;**31**:2391–7.
86. Everhart J, Wright D. Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. *JAMA* 1995;**273**:1605–9.
87. Huxley R, Ansary-Moghaddam A, Berrington de Gonzalez A, Barzi F, Woodward M. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. *Br J Cancer* 2005;**92**:2076–83.
88. Bonovas S, Filiooussi K, Tsantes A. Diabetes mellitus and risk of prostate cancer: a meta-analysis. *Diabetologia* 2004;**47**:1071–8.
89. Kasper JS, Giovannucci E. A meta-analysis of diabetes mellitus and the risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:2056–62.
90. Martin-Castillo B, Dorca J, Vazquez-Martin A, et al. Incorporating the antidiabetic drug metformin in HER2-positive breast cancer treated with neo-adjuvant chemotherapy and trastuzumab: an ongoing clinical-

- 
- translational research experience at the Catalan Institute of Oncology. *Ann Oncol* 2010;**21**:187–9.
91. Muti P, Berrino F, Krogh V, et al. Metformin diet, breast cancer: an avenue for chemoprevention. *Cell Cycle* 2009;**8**:2661.
92. Cazzaniga M, Bonanni B, Guerrieri-Gonzaga A, Decensi A. Is it time to test metformin in breast cancer clinical trials? *Cancer Epidemiol Biomarkers Prev* 2009;**18**:701–5.