

METFORMIN: CURRENT CONCEPTS AND FUTURE PERSPECTIVES

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ABSTRACT

Metformin, a biguanide is a preferred oral hypoglycemic agent for type 2 diabetes. It has also found to be beneficial in delaying the occurrence and progression of complications of diabetes Metformin is also being used as an off label drug for polycystic ovarian syndrome, antipsychotic induced weight gain, obesity and gestational diabetes mellitus. Research to study its role in malignancies is ongoing. The pleiotropic effects of metformin definitely will lead to multiple indications for metformin in the near future. This review focusses on the current status and future perspectives of metformin.

KEY WORDS: Metformin, hypoglycemic agent, weight gain.

INTRODUCTION

Metformin (1,1-dimethylbiguanide), a guanidine derivative is an oral hypoglycaemic agent belonging to the biguanide class of drugs. Metformin is the active glucose lowering constituent of the French lilac plant (*Gallegaofficianalis*) which was used in Europe as treatment for diabetes for centuries. Phenformin was the first biguanide to be introduced in the 1950's, but was withdrawn from the markets due to the serious adverse effect of lactic acidosis³. In 1990's metformin became the most widely prescribed drug for type 2 diabetes mellitus after receiving approval from the US-FDA⁴. It is the most commonly prescribed drug for the treatment of type 2 diabetes mellitus¹. According to American Diabetes Association guidelines, Metformin is the preferred initial pharmacological agent for type 2 diabetes, because (1) its main action is to decrease hepatic glucose production rather than increase insulin secretion, making it especially useful in insulin resistance, (2) its prolonged use reduces incidence of complications of diabetes, (3) it is safe on long term use, (4) it is less

expensive, (5) adverse effects like hypoglycaemia and weight gain are not seen.² However in spite of its extensive clinical use, its mechanism of action still remains unclear. This review aims to focus on the current status and the future perspectives of metformin. An energy deprived state characterized by deficiency of ATP by binding of metformin to complex I of electron transport chain is the mechanism for its anti-hyperglycaemic effect. The energy deficient state can lead to actions which may be AMPK dependent and independent². [A] AMPK dependent mechanism AMPK (AMP dependent protein kinase) is a serine-threonine kinase that acts as a monitoring system that conserves cellular function in energy deprived states and enables organ systems to function optimally in this state without shutting off.

AMPK activation in the liver results in

1. reduced hepatic glucose production by increasing degradation of transcriptional co-activator of CREB (TORC2), upregulating (short heterodimer protein) SHP and degrading KLF15 (Krupel like factor 15) [see FIG1] The above actions result in inhibition of CREB dependent PGC-1 α expression, thereby reducing the transcription of gluconeogenic enzymes, phosphoenol pyruvate carboxykinase (PEPCK) and glucose 6phosphate dehydrogenase².
2. decreased lipogenesis and increased lipid oxidation due to downregulation of transcription factors for lipid synthetic pathways resulting in inactivation of acetyl coA carboxylase (ACC), the rate limiting enzyme in biosynthesis of fatty acids and potent inhibitor of mitochondrial fatty acid oxidation; also decreased expression of fatty acid synthase gene, the key enzyme for de-novo fatty acid synthesis².
3. reduced food intake and early satiety which could be due to increase in GLP-1 levels⁶.

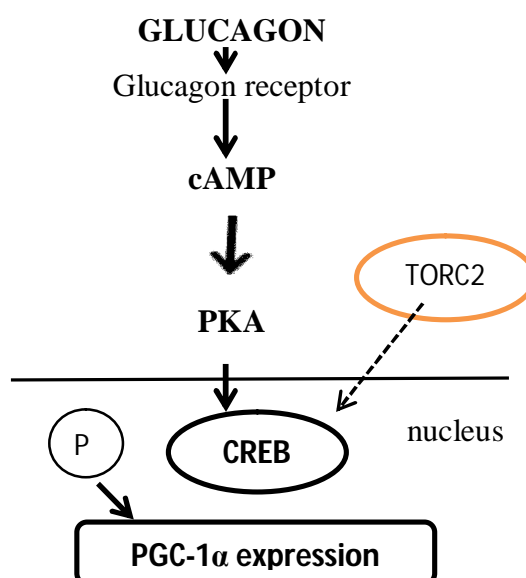


FIG1: Induction of gluconeogenesis by glucagon

When glucagon binds to its receptors there is activation of protein kinase A (PKA) which causes phosphorylation of cAMP response element binding protein (CREB). For further actions CREB requires transcriptional co-activator of CREB (TORC2), a key regulator in activation of gluconeogenesis. TORC2 translocates from the cytoplasm into the nucleus and together they increase transcription of PPAR γ coactivator 1 α (PGC-1 α) gene expression and thus increase gluconeogenic enzyme synthesis.

SHP prevents the CREB –TORC2 complex from carrying out its activities

KLF15 increases gluconeogenesis in response to hormones like glucocorticoids.

AMPK activation in skeletal muscles and adipose tissue

1. reduces postprandial hyperglycemia by activation of atypical protein kinase C (aPKC) which increases the translocation of GLUT-4 to the plasma membrane of these organs, thereby improving glucose uptake.
2. There is also a decrease in the lipid content by mechanisms similar to the ones operative in the liver.
3. Additionally, in the adipose tissue, the glucose that is taken up is diverted towards formation of glycerol phosphate which is utilized in re-esterification of fatty acids and stored as adipose tissue. This has two advantages; 1) there is decreased lipolysis and by that decrease in the levels of circulating LDL and VLDL, and 2) as glycerol phosphate is used for adipose generation less glycerol is available for gluconeogenesis, thereby further decreasing the hepatic glucose output. All the above mechanisms help in improving insulin sensitivity⁷.

[B] AMPK independent mechanisms

Activation of AMPK is not entirely necessary for the metformin action. Through inhibition of oxidative phosphorylation, metformin brings about an ATP deficient state in the body which by itself is an inhibitory factor for all energy consuming processes in the body like gluconeogenesis which results in inhibition of gluconeogenic enzyme fructose 1,6 biphosphatase that converts fructose 1,6 biphosphate to fructose 6 phosphate. Thus the net effect is reduction in de novo glucose production and reduction in blood glucose levels.

The net summary of all the above effects is that due to normalisation of blood glucose there is concurrent decrease in the compensatory hyperinsulinemia due to pancreatic oversecretion².

Metformin in complications of type 2 diabetes mellitus

However well controlled diabetes is occurrence of complications like nephropathy and cardiovascular problems are almost inevitable. According to the UKPDS longitudinal trial, when metformin was used as primary prevention from the onset of diabetes there was a 42% reduction in diabetes related morbidities and death as compared to insulin and sulfonylureas at approximately a 10.7 year interval⁸.

[A] Cardiovascular benefits of metformin

Johnson et al. showed that metformin alone or in combination with other agents can reduce cardiovascular mortality and morbidity as compared to the secretagogues in patients with heart failure through AMPK dependent mechanisms⁹.

1. Myocardial preconditioning, a technique wherein repeated short episodes of ischemia protects the myocardium against a subsequent ischemic insult. It helps limit the infarct size after further attacks and also improves the preserved left ventricular ejection fraction.
2. Reduction in apoptotic cardiomyocytes due to hypoxia/ reperfusion injury by decreasing the pro-apoptotic proteins and increasing the anti-apoptotic proteins.
3. Adaptation of metabolism by shifting energy production from fatty acid oxidation to glucose metabolism in myocardium as fatty acid oxidation utilizes more oxygen per ATP produced.
4. Restoring dysregulated autophagy so that the dysfunctional infarcted myocytes undergo degradation thus reducing the incidence of heart failure due to cardiomyopathy².

The REACH registry study on diabetes patients with atherothrombosis showed that metformin may reduce mortality in subsets of patients in whom it is not currently recommended like those with history of congestive heart failure, old age etc¹⁰

5. Inhibiting cardiac hypertrophy by increasing nitric oxide production and decrease in angiotensin II induced protein synthesis responsible for negative remodelling of myocardium.
6. Inhibiting reactive oxygen species production by inhibition of NADPH oxidase and mitochondrial respiration, reduction in levels of ICAM-1, VCAM-1 and advanced glycated end products (AGE's), and induction of superoxide dismutase².

[B] Diabetic nephropathy

It is a known fact that at least 20-40% diabetics progress to nephropathy eventually. Metformin can retard the onset as well as progression of microvascular complications of diabetes like nephropathy. Through AMPK independent mechanisms it can cause a decrease in the TGF- β action, thus inhibiting the epithelial to mesenchymal transition in renal tubules. It also reduces expression of hypoxia inducible factor 1- α (HIF1 α), thus preventing tubular fibrosis. Through AMPK dependent mechanisms; 1) decrease in ROS production and hence less damage to podocytes, 2) decrease expression of lipogenic enzymes and hence, reduced lipotoxicity, 3) decrease in mTOR(mammalian target of rapamycin) activation, thus causing less cystic growth in polycystic kidney disease. Thus these mechanisms prevent tubular injury and development of tubulointerstitial kidney disease². However, its use is contraindicated in renal disease. But recent findings suggest that it may actually be beneficial in chronic kidney disease.

Off label uses of metformin

An additional importance of understanding the above mentioned mechanisms of action of metformin is that it also helps in understanding its use in a variety of other conditions where it is prescribed as an off-label drug. Some of these conditions are; polycystic ovarian syndrome (PCOS), obesity, antipsychotics induced weight gain, gestational diabetes mellitus, etc.

[A] Polycystic ovarian syndrome (PCOS)

PCOS is the most common endocrinopathy affecting 5-15% women⁷. Diagnosis is confirmed by presence of features like menstrual disturbance, hyperandrogenism and polycystic ovaries on USG². Figure 2 explains the possible mechanism of anovulation in PCOS¹¹. In addition to this, the hyperinsulinemia which is commonly seen in women with PCOS can worsen the hyperandrogenism by

increasing the synthesis of steroidogenic hormones like CYP17 (17 α hydroxylase, 17 20 lyase), 3 β HSD, P450 side chain cleavage enzyme and StAR protein, thus increasing androgen production from the adrenals and ovaries as well.

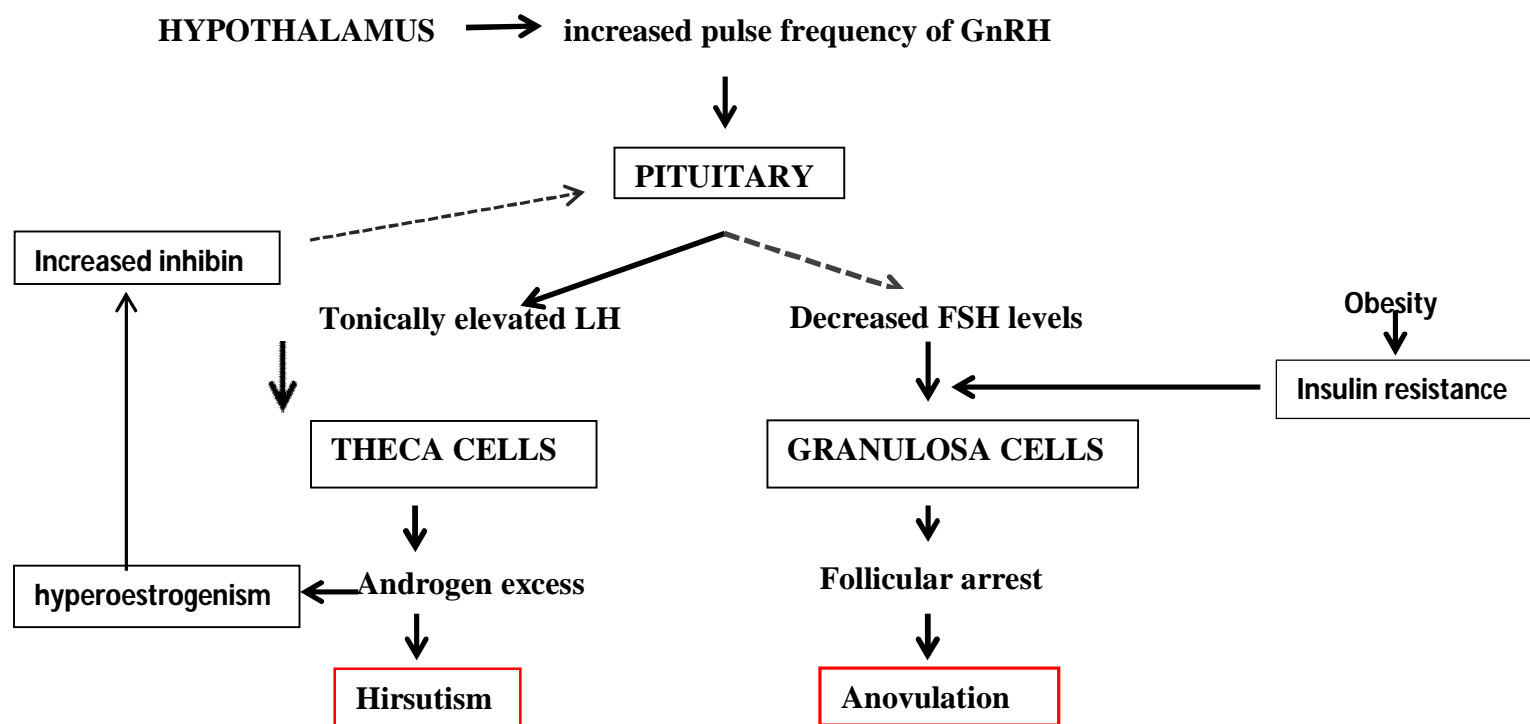


FIG2: Pathophysiology of PCOS

2)decreasing hepatic synthesis of SHBG (sex hormone binding globulin) ,thus increasing the levels of free circulating androgens. 3)decreasing synthesis of IGFBP (insulin like growth factor binding protein) and increasing levels of free IGF-1 which further stimulate the theca cells of ovaries to produce more androgen¹¹. A meta-analysis of 31 clinical trials showed that metformin benefits in PCOS by increasing ovulation, reducing serum androgen levels and improving menstrual cyclicity¹². Metformin exerts its effect by decreasing the excess insulin, as mentioned in the discussion on diabetes, as well as by direct ovarian actions. The reduction in insulin levels restores hepatic production of both IGFBP and SHBG, thus reducing free IGF levels and subsequent theca cells stimulation,and decreasing the free androgen levels respectively. By its direct action on theca cells it decreases the CYP 17 hormones synthesis and by causing AMPK activation it reduces the levels of the other steroidogenic hormones (StAR protein, 3 β HSD, etc). As a result of these effects metformin restores the GnRH regulation and ovulation in these women⁷. Not only does metformin help restore menstrual function and improve fertility in PCOS, it also helps in maintaining pregnancy in these women. By restoring IGF-BP levels, increasing glycodelin levels, a glycoprotein that protects the embryonic allograft and improving uterine blood circulation it favours implantation of the embryo. It also causes reduction in factors that may cause abortion like endometrial androgen excess, plasminogen activator inhibitor-1 and plasma endothelin².All these factors would make metformin a favourable option in the setting of PCOS and insulin resistance.

[B] Obesity

Insulin resistance is also a problem in obese individuals, even in the absence of diabetes¹³. The Diabetes Prevention Program, a randomised controlled trial showed that metformin when used in the doses between 500-2500mg in divided doses could achieve significant weight loss, even in non-diabetic individuals¹⁴. The mechanisms by which metformin is thought to bring about weight loss is as follows:

1. Improvement in the insulin sensitivity of peripheral tissues causes uniform uptake of glucose from blood and thus prevents slow post-prandial insulin secretion with a delayed peak. This helps in avoiding postprandial hypoglycaemia which consequently results in less carbohydrate craving and reduced food intake.
2. On prolonged use, there is decreased hepatic glucose output and reduced intestinal absorption of glucose. Hence, there is a deprivation of the energy source needed for re-esterification and storage of fat in adipose tissue.
3. Even in the setting of insulin resistance, the abdominal fat retains its sensitivity to insulin which enables it to take up and breakdown glucose and store fat in adipose tissue, thus being responsible for the centripetal obesity associated with obesity. Also the high peripheral glucose stimulates pancreas to cause hypersecretion of insulin to overcome this, leading to a vicious cycle. When there is improvement in insulin sensitivity in peripheral tissues, glucose is easily cleared from circulation and the natural outcome is reduction in pancreatic hypersecretion as well as less energy source to store fat in the abdominal region. This results in weight loss.
4. Metformin is also thought to have anorectic effects on the body and that may be mediated through increased GLP-1 levels.
5. Also there is a decrease in leptin levels which indicate an improvement in leptin resistance. Leptin released in response to fat accumulation in the body, stimulates receptors that increase energy expenditure in the body both by central as well as peripheral actions¹³.

The advantage of weight loss using metformin is that as compared to hypocaloric diet metformin causes reduction in only the adipose tissue formation and has no effect on the lean body mass¹³. Using metformin in non-diabetic individuals is also safe as it is seen that it doesn't adversely affect glucose levels in these individuals¹⁵. Metformin may thus be an inexpensive alternative for weight loss in obese individuals even in the absence of diabetes.

[C] Antipsychotics induced weight gain

Antipsychotics induced weight gain is also associated with metabolic abnormalities when used for a long duration¹⁵. A meta-analysis of controlled trials showed that metformin may prove useful in bringing about a moderate amount of weight loss (2.5-5 kg) in such antipsychotic users along with lifestyle modifications¹⁶. The mechanisms may be the same as those in obesity as insulin resistance is often seen as a result of metabolic derangements. Although effectiveness in reducing weight was not limited to people with insulin resistance but the results were found to be better and the reduction more significant in its presence¹⁵.

[D] Gestational diabetes mellitus

Obesity increases the risk of gestational diabetes mellitus and may also be associated with adverse outcomes². The Metformin in Gestational Diabetes Trial showed that the pregnant women treated with metformin had less weight gain as compared to those treated with insulin. There was no increase in adverse perinatal outcomes in the metformin group either. Women also preferred metformin over insulin considering the ease of administration¹⁷.

New therapeutic perspectives of metformin

Metformin is thought to be useful in both prevention as well as treatment of various malignancies. Diabetes associated hyperinsulinaemia and hyperglycemia are thought to be responsible for a wide array of malignancies. Metformin being the first line treatment option in type 2 diabetes has resulted in its concomitant administration with many anti-cancer drugs without any evidence of interactions. Therefore, metformin has bypassed phase I studies and directly moved on to phase II and III studies in cancer¹⁸. A recent meta-analysis showed a 31% reduction in the incidence of overall cancers and related mortalities when metformin was concurrently used for type 2 diabetes instandard doses of 1500-2500mg/day in adults¹⁹. The benefits are extended to a wide variety of cancers.

The most probable mechanisms by which metformin exerts its actions are

1. Activation of AMPK pathway,
2. Inhibition of protein synthesis,
3. Induction of cell cycle arrest,
4. Induction of apoptosis,
5. Reduced IGF-1, insulin, HER2 signalling,
6. Reduction in insulin levels²⁰.

For ease of understanding the above mechanisms can be further divided as; direct (insulin independent) and indirect (insulin dependent) mechanisms¹⁸.

[A] Direct (insulin independent) mechanisms

(a) Inhibition on mTOR signalling.

Protein synthesis, one of the major energy consuming pathways of the body is the main target of AMPK activation. Many cancers bring about protein synthesis by PI3K/AKT pathway activation which inhibits TSC2 protein (tuberous sclerosis) and prevents it from inactivating the RhebGTPase activity. The RhebGTPase prevents phosphorylation of Raptor protein which then associates with mTOR and leads to protein synthesis and cancer cell proliferation^{2,18}. (Fig 3)

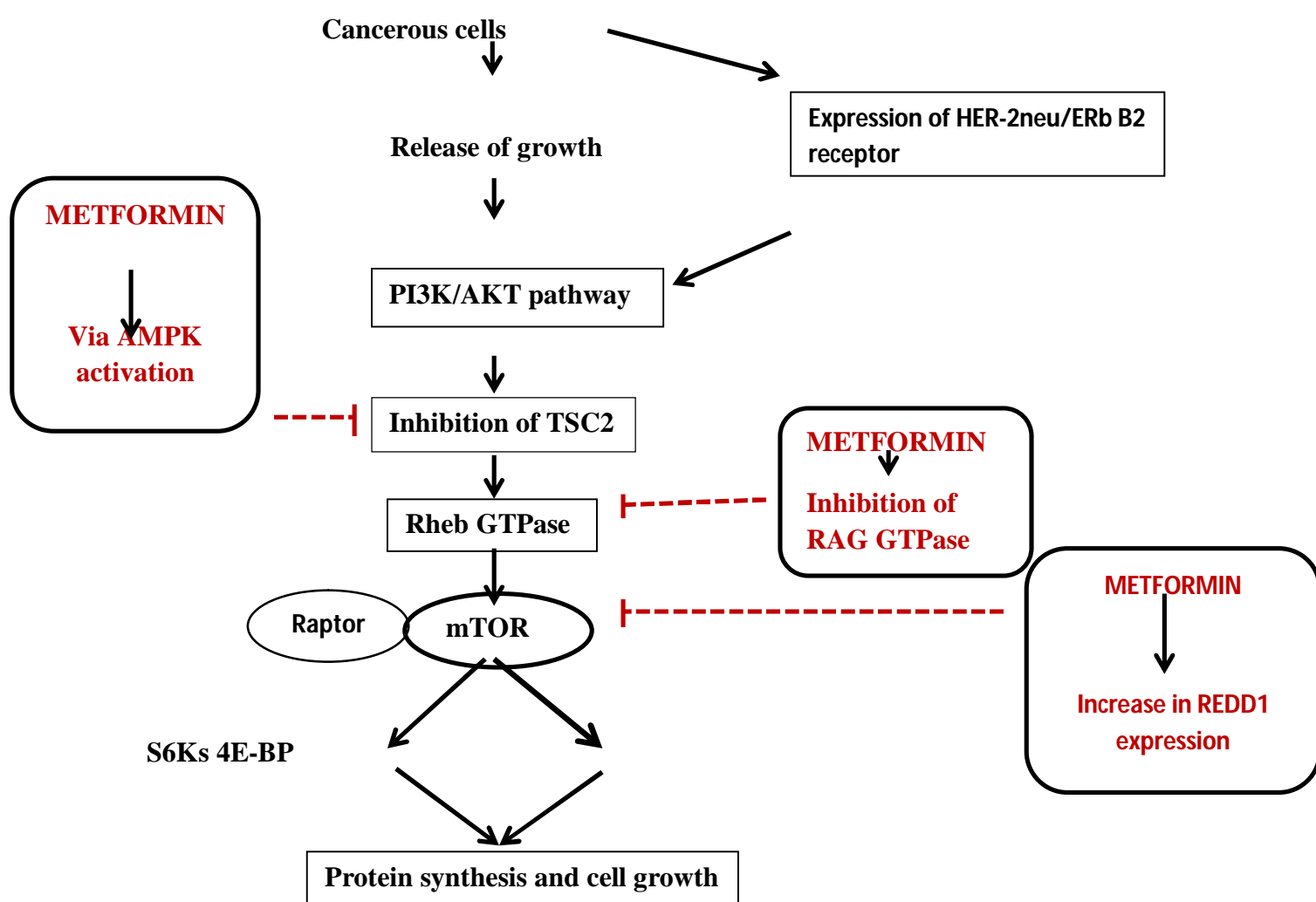


FIG 3: Protein synthesis by cancer cells through mTOR activation

Metformin via AMPK activation causes phosphorylation of TSC2 protein and prevents its inhibition, thus leading to inactivation of Rheb-GTPase and accumulation of Rheb-GDP. As a

result of this, the protein Raptor undergoes phosphorylation preventing its association with mTOR and inhibiting the downstream effects of mTOR. This is seen in breast, ovarian and colon cancers^{2,18}. Metformin can also produce its effects independent of AMPK activation, by direct inhibition of RAG GTPase, preventing the inactivation of other GTPases that inhibit Rheb GTPase¹⁸. It also increases expression of REDD1 via p53 regulation which is a gene causing apoptosis of cancer cells by inhibiting the downstream effects of mTOR activation. This REDD1 gene mediated action is seen in prostate cancer. Also decreased products of protein synthesis like protein p70s6K1, causes a decrease in HER-2 neu expression in breast cancers².

(b) Cell cycle arrest

In presence of DNA damage, p53 arrests cell cycle and gives the cell time to repair the damages. If the damages are irreparable it brings about apoptosis of cells. Certain cancers like colon cancer occur due to p53 mutations and loss of damage control leading to accumulation of mutations resulting in carcinogenesis²¹. In the colon cells, Wnt/ β -catenin pathway play a pivotal role in epithelial cell renewal and dysregulation of this pathway may lead to colorectal cancer. Normally, whenever there is any damage a signalling molecule Wnt binds to the frizzled receptors on the colon epithelial cells. The β -catenin (a molecule that upregulates cellular proliferation during wound healing) which is bound by E-cadherin to the cytoplasmic membrane gets released and translocates into the nucleus. Here it complexes with transcription factor (TCF) and increases expression of c-myc and cyclinD1, thus upregulating cellular proliferation and protein synthesis to repair damages. After its function is completed β -catenin undergoes phosphorylation and ubiquitination by APC (adenomatous polyposis coli) gene²². This APC gene is also regulated by p53²¹ (Fig 4). If there is a p53 mutation leading to dysregulation of the APC gene and loss of control over β -catenin degradation, it may result in excessive DNA transcription and protein synthesis by the β -catenin molecule and tumorigenesis²².

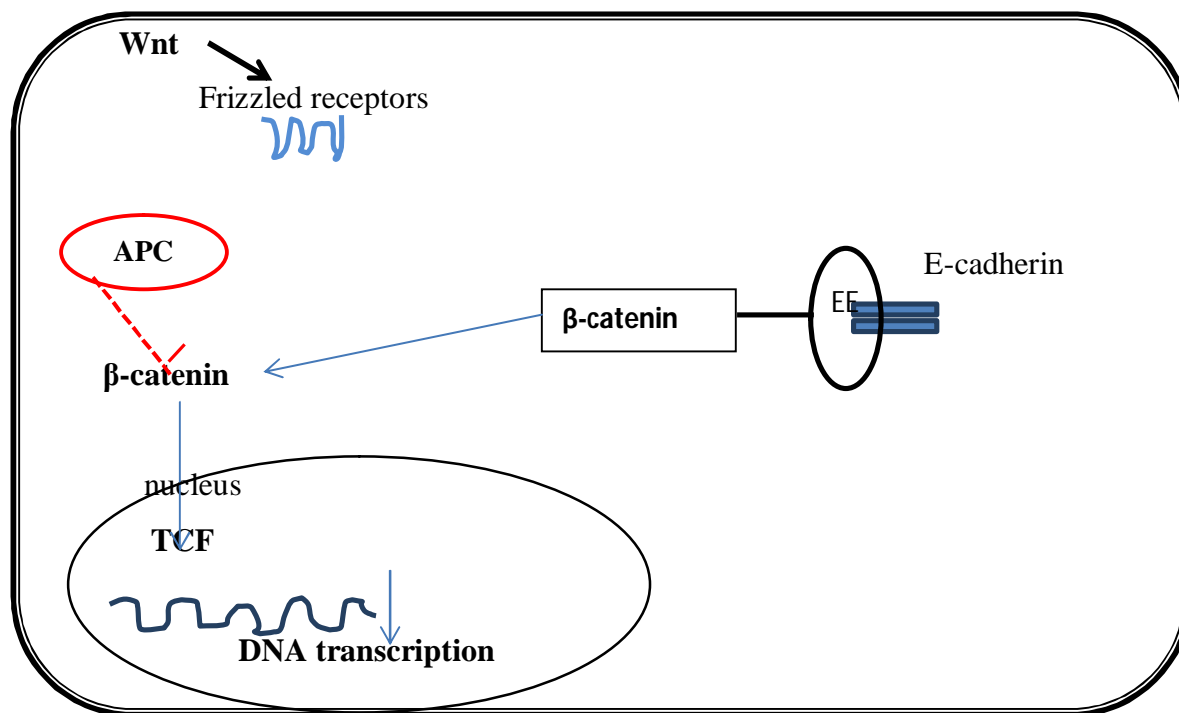


Fig. 4: Wnt/ β -catenin pathway and regulation by APC gene.

1. The therapeutic efficacy of metformin on colorectal cancer may be due to Inhibition of binding of Wnt to the frizzled receptors and thereby inhibiting β -catenin signalling, causing a cell cycle arrest,
2. Increasing sensitivity of cancer cells to FuOx(5-fluorouracil and oxaliplatin) chemotherapy,
3. Inhibition of migration of cancer by avoiding colonosphere formation (large,round unattached floating spheroid colonies),
4. Prevents recurrence in colon cancer²².

However, in prostate cancer p53 expression is intact. Here metformin decreases the levels of transcription factors and thereby decreases expression of cyclin D1 causing a cell cycle arrest¹⁸. Also since p53 is intact,it increases upregulation of REDD1 gene and inhibits mTOR signalling to reduce cellular proliferation and induce apoptosis of cancer cells².In breast cancers, metformin causes cell cycle arrest by increasing the binding of p27Kip and p21Cip proteins to CDK2,preventing cyclinD1- CDK2complex formation and inhibits the cell cycle from progressing. Also it causes downregulation of cyclin D1 expression².

(c) Apoptosis

Metformin can cause apoptosis of cancer cells by either p53 regulation in tumorswhere p53 function is intact as well as by caspase dependent and independent mechanisms²³.

(d) Other mechanisms

There may be a few additional mechanism of action of metformin in cancer cells which may not be entirely understood. It is found that metformin can cause eradication of cancer stem cell traits if used at an early stage by decreasing TGF- β levels and preventing epithelial to mesenchymal transition of these cells²⁴. It also inhibits angiogenesis in tumors by decreasing levels of VEGF and PAI-1. Inhibition of ROS production may also add to its anti-cancer mechanisms².

[B] Indirect (insulin dependent) mechanisms

Insulin and glucose exert their pleiotropic actions via PI3K/AKT activation leading to mTOR signalling and its downstream effects. Metformin reduces the circulating levels of insulin, glucose as well as free fatty acids as explained earlier, thus reducing their effects on cell growth and other effects. Also it reduces mTOR signalling as previously explained¹⁸.

Most of the above effects are studied in the setting of diabetes and the effect on non-diabetics cannot be foretold. A 'window of opportunity' trial done in breast cancer examining the effects of metformin in non-diabetic women with inoperable tumor at 1000 mg twice daily dose showed significant reduction in numerous genes involved in carcinogenesis²⁵. A RCT conducted in non-diabetic individuals with recent history of polypectomy showed that metformin had favourable effects in reducing proliferation and aberrant crypt formation, thus preventing recurrence in these patients²⁶. Thus some of the above mechanisms (the insulin independent) may also be applicable to non-diabetics with various cancers, but further studies may be needed to ascertain the same.

VII) Re-emerging uses of older biguanides

Phenformin was the first biguanide to be introduced into the markets, but it was withdrawn in 1970's due to high risk severe cases of lactic acidosis. Presently, it has been found in some in-vitro studies that phenformin may have a greater anti-cancer effects than metformin. This may be owing to various factors like increased lipophilicity making its entry into cells independent of carriers and, more potent inhibition of AMPK exhibiting higher cytotoxicity and growth suppression to cancer cells. To vitiate the risk of development of fatal lactic acidosis it could be combined with oxamate salts, a competitive inhibitor of the lactate dehydrogenase enzyme²⁷. If this combination finds results in further studies, we may see the re-emergence of the use of this withdrawn drug.

CONCLUSION

Metformin is currently a first line agent used in type 2 diabetes mellitus treatment. In controlling hyperglycemia and hyperinsulinaemia it shows a wide range of activity from switching off of oxidative phosphorylation to AMPK activation. It has also found to be beneficial in delaying the occurrence and progression of complications of diabetes. But its use not being limited to treatment of diabetes, this enterprising group of drugs have found application in other arenas like PCOS, weight loss, Gestational Diabetes mellitus etc. Since AMPK activation also has a great variety of downstream mediators, the effects of metformin are being tested in a variety of cancers. But even with this the potential of this drug seems to be so great that it is possible that what has been discovered may have just scratched the tip of the ice-berg. Many potential applications of biguanides are in the pipe-line like use in rheumatoid arthritis, psoriasis, non-alcoholic steato-hepatitis, elimination of cancer-stem cells, etc. Only further research can show which of these efforts will bear fruits.

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