Role of Metformin in Enhancing Cancer Therapy

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Abstract
Metformin is a lipophilic biguanide derivative, which is usually been considered an antidiabetic drug, many researchers have found an association between metformin and reduced risk of cancers. While, metformin acts primarily on decreasing liver glucose synthesis thus, reducing the concentration of blood glucose and affecting a variety of biological pathways, either activating AMP-kinase-dependent pathways or non-AMPK-dependent pathways like, insulin-like growth factor-1 and inflammatory pathways. Emerging evidence from large-scale observational and cohort studies suggests that metformin may be useful as an adjuvant agent, with the greatest benefits in the prevention and reduced risk of a number of cancers. Therefore, it was necessary to clarify the real role of metformin to be vital anti-tumor therapy. In this review, we will explain in detail the relationship between metformin and its use as a promising treatment against cancer, and we will present the relevant scientific evidence that mentioned the efficacy and advantages of metformin as a future therapy in oncology.
Keywords: Cancer, Metformin, Type 2 diabetes mellitus, Mammalian Target of Rapamycin

1. Introduction

Cancer (malignant neoplasm) is a worldwide disease characterized by metastatic and invasion capacity, absence of differentiation, and increased proliferation (American Cancer Society, 2015). It is considered the most common cause of death and morbidity worldwide, both in developed and developing countries. According to incidence rates in 2022, 1.918.030 cases were registered as newly diagnosed while 609.360 deaths. (American Cancer Society, 2017).
The incidence of cancer globally and locally increased, for example, 11559 new Jordanian cases were registered in 2020 (Al-Sayaideh et al., 2012). Whereas in 2017 the total number of new cases was 8755 cases, 6352 of them were Jordanian and 2403 cases were non-Jordanian. In 2021, the incidence rate of mortality was 15.7%, which is approximately 3084 deaths(Jordan Cancer Registry, 2021).
However, many studies suggested that diabetes patients might increase the risk of various tumors 1.41 times compared to patients without diabetes where, the pathological process of diabetes-related tumors involves several factors such as hyperglycemia, DNA damage, hyperinsulinemia, IGF 1, obesity, and inflammatory factors.
Accurate early diagnosis and screening of cancer patients is essential for selecting the appropriate program for effective treatment, which in turn can result in a less incidence of morbidity and mortality (Debela et al., 2021).
There are several routes to treat cancer like the use of radiation, chemical therapy, hormonal therapy, and biological therapy, or we can resort to medical surgeries and this depends on the type and stage of cancer (Amadori et al., 2018).
Over time, many medical drugs and treatments have been used, and research has proven effective in contributing to the treatment of cancer (Rizos & Elisaf, 2013). Metformin is one of the medications that exhibits effective properties as adjuvant therapy in cancer treatment (Evans et al., 2005).
New evidence from some studies submits that metformin, a generally used antidiabetic drug, may help prevent and treat cancer (Skuli et al., 2022). Therefore, in this review, we amid to demonstrate the relation between receiving metformin and the prevention of the risk of various types of cancer, and the efficacy of metformin as an anti- proliferation drug in cancer according to several reviews and manuscripts.

2. Historical perspective of metformin

Metformin (Glucophage) is a biguanide anti-hyperglycemic agent; it returns discover in 1922, and in 1950 was studied in humans by French physician Jean Sterne (Bailey & Day, 1989). Moreover, it was the most generally recommended oral medication in the world (Viollet et al., 2012). The main role of metformin is decreasing hepatic glucose production, in many ways like increasing insulin sensitivity, and increasing GDF15 secretion, which in turn lowers the appetite and caloric intake (O'MARA' et al., 1985) .
In 1958, the UK was licensed to use metformin as an oral antidiabetic drug, whereas the USA licensed it in 1995 because of worries about cardiac deaths and lactic acidosis (Dowling et al., 2011)[1].
Over time, metformin became safer, and the occurrence of lactic acidosis was very rare; in addition, many studies said that there is no relation between metformin and the risk of lactic acidosis (Sr et al., 2010). Although, there are some circumstances where increased the risk of lactic acidosis like tissue hypoxemia such as in acute kidney disease, hepatic, cardiac disease, and sepsis and this may be a

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particular link in patients with acute cancer illness (Zelenko & Gallagher, 2014).

Nowadays, metformin is supported to be the first therapy choice for diabetic patients (T2DM), based on several studies, which showed its effectiveness to lower the incidence of death in obese patients with T2DM. Despite that, it is not suitable for patients with kidney disease stage 4 (glomerular filtration rate (eGFR) less than 30 mls/min/1.73m2). So, the recipe of metformin for patients with chronic kidney disease must be ordered according to some illness rules (Adam & Brien, 2014).

Furthermore, metformin has multiple functions, for example, it has avital role in protecting cardiovascular system, also it can be used as adjuvant therapy in tuberculosis, and as routine treatment in polycystic ovary syndrome[2].

Additionally, metformin exhibits anti-aging effect and can be used to prevent and treat uveitis, improve the symptoms of non-alcoholic fatty liver, improve the intestinal flora imbalance in diabetic patients, and reduce the prevalence of Parkinson's disease.

3. Anticancer mechanism of metformin

2.1 The role of metformin in cancer

However, using metformin combined with another drug like risperidone in patients with schizophrenia can improve triglyceride levels, high-density lipoprotein levels (HDL), fasting blood glucose, and body mass index[3].

Metformin has several effective properties in cancer therapy, which affect cell cycle, proliferation, or induction of cell death (apoptosis) (Skuli et al., 2022) (Ejaz et al., 2020). Moreover, it could stimulate other ways of cell death, like caspase, 1-dependent programmed cell death, or induce pyro- ptosis (cell inflammation) by inducing the LKB1/AMPK pathways (adenosine monophosphate-activated protein kinase) (Green et al., 2016).

Interestingly, metformin appears effective role on insulin and IGF-1 whose receptors are found on many cancer cells like the colon, skin, breast, and pancreas. In in-vivo studies showed that metformin decreases the level serum of insulin and IGF-1, which inhibit growth stimulation, furthermore, in vitro research showed that retraction of glucose may increase apoptosis (cell death) (Heiden et al., 2001).

Importantly, the mechanism of action of metformin acts to induce the secretion of glucagon-like peptide-1 (GLP-1), and beta-cell GLP-1 receptor expression, by mediated pathway peroxisome proliferator-activated receptor-α (PPAR-α) (Maida et al., 2011). Also, metformin reveal anti-cancer effect on several key genes and their protiens, for example, B-cell CLL, mitogen-activated protein kinase ½, and activator of transcription 3. Table (1).

Table (1) anti-cancer properties of metformin on key genes/protein expression.

Gene/protein	Form of effectiveness
Mitogen-activated protein kinase (MAPK)	Down-regulation
B-cell CLL/lymphoma 2 (BCL-2)	Down-regulation
RAC-alpha serine/threonine-protein kinase (AKT)	Down-regulation
Mitogen-activated protein kinase p38 (P38)	Down-regulation
Cyclin-dependent kinase inhibitor 1B (P27)	Up-regulation
Ribosomal S6 kinase (RSK1)	Up-regulation
Signal transducer and activator of transcription 3 (STAT3)	Down-regulation
Mammalian target of rapamycin (mTOR)	Down-regulation
Mitogen-activated protein kinase ½ (ERK1/2)	Up-regulation
AMP-activated protein kinase (AMPK)	Up-regulation
Glioma-associated oncogene homolog 1 (GLI1)	Down-regulation
c-Jun N-terminal kinase (JNK)	Down-regulation
Excision repair cross-complementation 1 (ERCC1)	Down-regulation
Dual specificity mitogen-activated protein kinase ½ (MEK1/2)	Up-regulation
Thymidine phosphorylase (TP)	Down-regulation

The main action site of metformin in the cell is the mitochondria, mitochondrial membrane has a negative charge, which interacts effectively with the positive charge of metformin, causing a decreased level of cellular adenosine triphosphate (ATP) by inhibiting complex I of the mitochondrial electron transport chain and decreased oxidative phosphorylation, which increases the adenosine monophosphate (AMP)/ATP ratio within the cell, cellular stress, activation of AMPK, and downstream inhibition of mTOR kinase activity, which conclude to decrease in protein synthesis, cell growth, and proliferation (Figure 1) (Krishan. et al., 2014).

The activated protein kinase (AMPK) pathway has been shown a critical role in cell homeostasis, for example in a reduced energy situation the AMP/ATP ratio will increase and activate the AMPK by phosphorylation of Thr172 residue on α-subunit through some enzymes like calcium/calmodulin-dependent protein kinase (CaMKK), and liver kinase b1 (LKB1). This leads the cell to uptake more fatty acid and glucose and inhibits the synthesis of protein, glucose, and lipid, thus maintaining the energy balance of the cell.

2.2 AMPK activation pathway

The AMPK pathway is one of the important pathways that regulate several processes in the cell, especially in the pathogenesis of cancer (Faubert *et al.*, 2015). As mentioned above, metformin directly affects

cellular proliferation via consequent adjustment pathways (Daugan *et al.*, 2016).

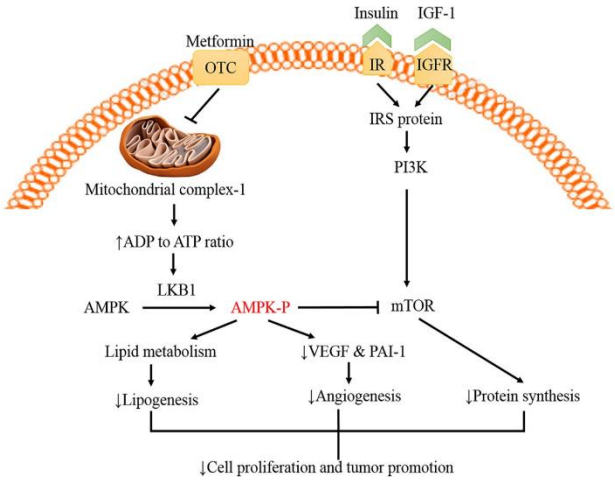

Figure 1: Molecular mechanism of action of metformin in cancer (Ejaz et al., 2020).

Metformin shows the activation of the AMPK pathway by inhibiting cell division in the mitosis stage by inhibiting the expression of cyclin D1 protein causing G1-phase arrest of the cell cycle, thus inhibiting cellular proliferation (Vakana et al., 2011).

Several studies have proven that activating the AMPK pathway leads to inhibits fatty acids synthase and acetyl CoA carboxylase (lipogenic enzymes), which are needed by the cancer cell to produce energy primarily by anaerobic glycolysis, called the Warburg effect (Lettieri et al., 2014).

In addition, activation of the AMPK pathway has several other effects like anti-inflammatory which decrease the pro-inflammatory cytokines, for example, interleukin-6 and 8 (IL-6, IL-8), tumor necrosis factor-alpha, and vascular endothelial growth factor (VEGF) (Nose et al., 2015). Therefore, increasing production of interleukin-6 induced signal transcription protein (STAT), resulting in cell proliferation, thus, decreased IL-6 cause inhibition of cellular proliferation (Yu et al., 2016).

2.3: Mammalian target of Rapamycin (mTOR) inhibition pathway

Several studies showed the critical role of the mTOR pathway in maintaining energy homeostasis in the cell in addition to angiogenesis via adjusting hypoxia-inducible factor 1α (HIF-1α) and VEGF. Metformin acts to decrease VEGF, plasminogen activator inhibitor-1 (PAI-1), and HIF-1α, which are all considered potent angiogenic stimuli in tumor cells (Hay & Sonenberg, 2004).

Activation and overexpression of the mTOR pathway are associated with several diseases like neurodegeneration, diabetes, and cancer (Zoncu *et al.*, 2011). Also, it responds to various stimuli like growth factors, cellular energy, and nutrients which regulate protein synthesis and cell proliferation (Bao *et al.*, 2019).

In cancer, there are various types, including leukemia, lung, breast, and thyroid cancer which revealed inhibition of mTOR by using metformin, additionally metformin activates the AMPK pathway which causes inhibition of the mTOR pathway through decreasing levels of insulin-like growth factors, and protein (Sp) transcription factors (Stephenne *et al.*, 2011) ;(Sachdev & Yee, 2007).

2.4 Insulin and Insulin-like growth factors pathway

Importantly, metformin shows acritical role in decreasing levels of insulin and insulin growth factor, which have anti-cancer properties (Pisani, 2008). As metformin indirectly reduces IGF-1 levels by decreasing levels of plasma insulin and insulin-binding proteins (Key et al., 2010).

Several studies conducted on rodent models indicated that metformin reduces cell proliferation and progress by decreasing levels of insulin and IGF-1 (Ma et al., 2010).

Moreover, metformin reduces the phosphorylation of insulin receptor substrate-1 (IRS-1) by activation of the AMPK pathway, which leads to inhibition of the mTOR pathway. Also, metformin is involved in the distribution of glucose transporters (GLUT-1) and (GLUT-4) from intracellular to the cell membrane, for example, an increased level translocation of GLUT-1 in the plasma membrane enhances the uptake of deoxyglucose (Abo-Elmatty et al., 2017).

2.5 Other signaling pathways.

There are a number of signaling pathways involved to regulate apoptosis, cellular growth, and proliferation like; Ras/Raf/Mitogen-activated protein kinase (MAPK).

The anti-cancer effect of metformin was revealed through activation of apoptosis, during activation MAPK pathway, DNA damage-inducible gene 153 (GADD153), and increased expression of growth inhibition. Interestingly, the anti-cancer mechanisms of metformin involve many transcription factors, like Sonic hedgehog, Kruppel-like factor 5, Wnt, mitogen-activated protein kinase (MAPK), Notch, and forkhead box O3a (FOXO3a) which support mitochondrial metabolism by its ability to enhance MAPK-dependent expression of the mitochondrial genome. Therefore, several multiple tumor models indicated that FOXO3a activation is very important for metformin effect (pro-apoptotic and chemosensitizing) by inducing the biogenesis of mitochondria and reducing complex I activity. (Wu et al., 2011).

Metformin appears to reduce nuclear factor kappaβ (NF-kβ) expression, which is a transcription factor that is involved in the regulation of cellular proliferation and apoptosis. Reducing NF-kβ may lead to decreased tumor cell proliferation (Ang et al., 1999).

Despite the AMPK/mTOR pathways are the most essential mechanism that exhibits the metformin anti-cancer properties, there are further pathways such as RAG GTPase, DNA response 1 (REDD1) expression, and Human epidermal growth factor receptor-2 (HER2) (Vazquez-martin & Menendez,

2011).

Role of metformin in the prevention of carcinogenesis risk

There are several evidence indicated that metformin has reduction role in the risk of various cancer types, whether a small dose or large dose used (1.500-2250 mg per day), for example, metformin appears to supress the proliferation of prostate, glioma, uterine,breast, ovarian, and colon cancer cells.

The anti-proliferative effects of metformin on cancer cells are associated with many pathways like AMPK activation, inhibition of the mTOR mechanism, decreasing of epidermal growth factor receptor (EFGR) and mitogen-activated protein kinase (MAPK), decreasing in the cyclins and p27gene expression and activity (Anisimov et al., 2005).

Also, metformin may have an important role in inhibiting cancer stem cells (CSCs), which are characterized by indefinitely renewing to form a cancer cell, and it is resistant to chemo and radiotherapy, also it showed diverse targeting of mitochondrial respiration in osteosarcoma stem cells, organization of transcription factors, and suppression of stem cell markers like CD133 in HCC and oral cancer cell lines and CD47 in breast cancer. (Hirsch et al., 2010). Metformin appears to have a role in improving cell response to chemotherapy via inhibiting and removing CSCs in various types of cancers by activating the AMPK pathway and inhibiting the mTOR cascade.

In addition, metformin has been postulated as having immunomodulatory effects on tumor cells, for example, it stimulates the CD8+ tumor-infiltrating lymphocytes (TILs) which leads to a cytotoxic response against cancer cells. Immunomodulatory, metformin inducing apoptosis and inhibits proliferation and cytotoxicity through activation the AMPK pathways and inhibition the mTOR pathways which are detrimental to CD19-chimeric antigen receptor-modified T cells. (Eikawa et al., 2015).

Interestingly, metformin has several roles in immune system cells for example, in cervical cancer, it promotes the cytotoxicity of natural killer (NK) cells through the PI3K/AKT pathway it can alter the expression of cancer cell surface ligands which leads to an increase NK cell activation. also, metformin affects the expression of macrophage related cytokines, depending on AMPK/NF-κB pathway so, it enhances the anti-cancer macrophage 1 phenotype and reduces the potency of cancer cells to enhance the protective macrophage 2 phenotype. Furthermore, meformin exhibites a critical role Epigenetic action like methylation of cancer suppressor genes, demethylation of general genome, and alteraing in posttranslational modifications of histone which play importany role in oncogenesis.

Recent studies showed several roles of metformin via epigenetic modifications, such as increased methylation of global DNA in various types of cancer like breast, colon, and endometrial cancer. also, regulation of long non-coding RNAs by Altered DNA methyltransferase (DNMT) activity. moreover, many studies indicated the main role of metformin through targeting oncometabolite 2-hydroxyglutarate (2HG) either by the traditional route of targeting IDH1/2 mutations or knockdown of phosphoglycerate dehydrogenase in the AMPK-dependent pathway which lead to anti-cancer activity.

Also, metformin suppresses the DNA damage via activation of Ataxia Telangiectasia Mutation (ATM) which consider an essential component of DNA repair and inhibits reactive oxygen species (Vazquez-martin et al., 2011). Moreover, metformin appears to have suppressed effect on epithelial-mesenchymal transition (EMT) a biological process that is critical to metastasis formation, especially in breast and colorectal cancer (Y. Wang et al., 2017). In colon cancer patients treated with metformin showed a decreased growth of tumor cells and destruction of aberrant crypt foci (ACE), which is an important marker related to presence of colon cancer. Furthermore, the use of metformin decreased the probability of death among colorectal cancer patients by 40% from grade one to four, whereas in prostate cancer which considered the second leading cause of death among men, metformin decreased the chance of recurrence of disease by 18% and improve some indicators like CSS, RFS, and OS.

Generally, according to clinical trials and related articles we conclude that metformin has a potential role in decreasing the risk of cancer, especially in diabetic patients, with various probability depending on tumor type, patient characteristics history, tumor biology (Figure2).

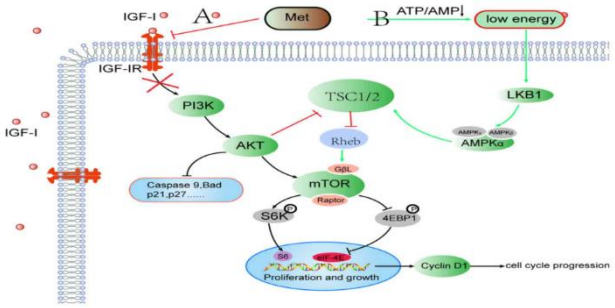

Figure 2: Anti-cancer effect of metformin (Zi et al., 2018)

Metformin in cancer treatment

Cancer therapy is the regime used to reduce tumor growth, relieve signs and symptoms, prevent the risk of cancer recurrence, or improve adjuvant therapy effectively (Rizos & Elisaf, 2013). Abundant animal studies in vitro and in vivo have indicated the metformin inhibiting effect on growth, especially in breast, lung, endometrial, liver, gastric, and thyroid cancer cell lines (Viollet et al., 2012). Furthermore, there are anti-proliferative effects on hemopoietic cancer cells like promyelocytic leukemia cells and acute myeloid leukemia (Hirsch et al., 2013).

Several cohort studies reported an association between metformin and improved cancer survival, in

a prospective cohort study in the Netherlands that involve 1,353 T2DM patients' metformin showed a 57% reduction in cancer-specific mortality (Landman et al., 2010). Whereas a retrospective study conducted on 10,309 Canadian showed a 20% reduction in cancer mortality in new users of metformin or sulfonylureas compared with sulfonylurea monotherapy users (Bowker et al., 2010). However, in the United Kingdom, a subsequent study reported a 15% reduction in cancer mortality among metformin-treated patients (Currie et al., 2012).

While (Franciosi et al., 2013) reported a 35% reduction in cancer mortality among 28,671 metformin-treated patients. Otherwise, metformin users from randomized controlled trials were not reported any significant reductions in cancer mortality.

Interestingly, metformin indicated increased sensitivity of cancer cell lines treated with metformin to chemotherapy (Dong et al., 2012). These effects are due to growth suppression by cell cycle arrest or inducing apoptosis via increased cytotoxicity (Morales & Morris, 2015).

Recent reports demonstrated the synergistic activity of metformin with several other drugs like cisplatin, carboplatin, doxorubicin, and paclitaxel on various cancer types such as ovarian cancer cell lines, endometrial, and breast (Asensio-López et al., 2011) (Chang et al., 2014). Moreover, xenograft experiments suggest the role of metformin in protecting from several side effects of chemotherapy like doxorubicin-induced cardiotoxicity and cisplatin-induced ototoxicity (Jones & Zweier, 2014). For example, in non-small cell lung cancer treatment metformin shows more synergistic activity with tenovin-6 and appears more effective in suppressing cell proliferation and SIRT1. the researchers noticed that using a certain concentration of metformin with tenovin-6 (10 mmol/L metformin and 10 μmol/L tenovin-6) decreased cell growth in NSCLC cell lines (Lee et al., 2019).

In the next section, we will discuss various randomized and observational studies of metformin in the treatment of various cancer types.

4.1 Breast cancer

Molecular studies have effectively revealed some mechanisms of metformin, AMPK signaling pathway is considered the main pathway in decreasing cancer cells in the breast. In vitro studies have demonstrated the role of metformin in the up-regulation of miR 200c expression, which suppresses the growth and invasiveness of breast cancer cells, also metformin affects PI3K/Akt/mTOR MAPK pathways by inhibiting the mitochondrial respiratory chain complex I. While metformin play important role in modulation of the macrophage-targeting tumor cells, it suppresses the expression of CD47 gene in miRNA-708 to facilitate phagocytosis of breast cancer stem cells (Zhang et al., 2017).

Rendering to the results of many studies correlated to breast cancer, it has been shown that metformin can improve RFS, increase pathologic complete remission (pCR), and decrease the risk of breast cancer in individuals reciveing metformin for more than five years. Although, a recent study conducted on transgenic mice revealed the role of phenformin in suppressing the growth of breast cancer cells by targeting the IGF/IGF1 pathway and overexpressing ErbB2 (Guo et al., 2017).

Otherwise, studies conducted on a large retrospective population have not demonstrated any relationship between metformin and the stage or type of breast cancer. In the same study, the authors failed to show the association between metformin and mortality in breast cancer patients (Lega et al., 2017).

In 2015, a meta-analysis study showed a reduction in breast cancer mortality among metformin-treated patients (HR 0.652; 95% CI 0.488- 0.873; p=0.004). However, no relation between decreasing incidence of breast cancer in patients treated with metformin (Yang et al., 2015).

A subsequent study restricted to 1,983 patients with HER2+ breast cancer, showed better results when using metformin with a thiazolidinedione. While triple-negative breast cancer patients (who do not express estrogen or progesterone receptors, nor HER2 cell-surface receptors), showed diverse results (He et al., 2012). Although, it has been reported metformin can be used in chemotherapy as an adjuvant, where it works on reverse doxorubicin multidrug resistance (Shafiei-Irannejad et al., 2016).

A study of 1,031 diabetic breast cancer patients, showed that treated patients with metformin have better survival rates of about five years when compared with other patients, and showed a significant decrease in recurrence (Franciosi et al., 2013) (Luthar et al., 2013).

4.2: Lung cancer

There are several in vitro studies conducted to demonstrate the potential role of metformin in patients with lung cancer, according to their results we found that metformin has potential anti-tumor effects in various cell lines, for example, WA-hT, RERF-LC-A1, A549, and IA-5 cell lines models when exposed to a certain concentration of metformin equivalent 1 to 20 mmol, resulted in serious inhibition of cell growth as well as enhancing programmed cell death (apoptosis), and induce cell cycle arrest at G0/G1 phase (Lin *et al.*, 2018).

Non-small cell lung cancer (NSCLC) studies both in vivo and in vitro have been demonstrating that metformin enhances programmed cell death (apoptosis) and decreases the proliferation of lung cancer cells either in monotherapy or combination therapy (Yousef & Tsiani, 2017).

Furthermore, the role of metformin therapy in combination with other drugs was indicated in several studies, for example, using 1 to 10 mmol of gefitinib in combination with 0.1 to 1 mmol of metformin showed potential cytotoxic effects (Sayed

et al., 2015).
additionally, metformin has been shown as an anti-proliferative drug when used 20 mmole in combination with 20 mmole of β-element in PC9 and A549 cell lines, the author indicated that metformin improves the effect of β-element through knockdown the Akt signaling and suppress the expression of DNMT1 (Morgillo *et al.*, 2017).
As similar, the real action of metformin in patients with lung cancer has been demonstrated in several studies, for example from 2009 to 2013 a systemic study found that patients treated with metformin showed a decreased risk of lung cancer. while in a similar study the author reported that metformin can lower the risk of lung cancer by 39%–45%. (Nie *et al.*, 2014).

4.3: Pancreatic cancer

Oliveria et al and Ruiter et al reported that using metformin reduces the risk of pancreatic cancer either used alone or combined with another drug like sulfonylurea (Garrett et al., 2012).
In rodent models, metformin therapy has shown a decreased effect in acinar to ductal metaplasia and intra-epithelial neoplasia. Moreover, metformin reported an essential role in reducing the improvement of pancreatic cancer in meta-analyses of 11 observational studies (RR 0.63, 95% CI 0.46-0.86, p=0.003) (Z. Wang et al., 2014).
Otherwise, the addition of 2000 mg per day of metformin with standard therapy in metastatic pancreatic cancer failed to indicate significant improvement in DFS, OS, or PFS (Reni et al., 2016).
In meta-analysis of nine cohort studies and two RCTs was conducted to evaluate the survival rate of pancreatic cancer patients in all stages, which reported significant results in patients treated with metformin (HR = 0.86, 95% CI 0.76–0.97; P<0.05) and patients with resection and localized tumors (Li et al., 2017).

4.4: Colorectal cancer

In a Markov model analysis, T2D patients treated with metformin showed a lower rate of colorectal cancer (CRC) compared with non-treated patients and a significant statistically cumulative tumor-free survival (1.670% vs. 2.146%, P=0.016) (Mallik & Chowdhury, 2018).
A systematic review and meta-analysis of 10 studies, postulated that Colorectal adenoma is a precursor to CRC which reported the significant role of metformin in reducing the risk of colorectal adenoma (pooled OR = 0.61, 95% CI = 0.34-1.10, I2 = 79%) (Jung *et al.*, 2017).
Another study showed a decreased risk of colorectal adenoma in patients with metformin treatment (adjusted OR = 0.75, 95% CI: 0.59-0.97, p=0.03) and decreased risk of CRC (unadjusted OR=0.73, 95% CI: 0.62-0.86, p=0.0002) (Liu *et al.*, 2017).

4.5: Liver cancer

Metformin therapy showed a preventative agent for liver cancer and reduction of hepatocellular carcinoma proliferation, for example, a retrospective study conducted to compare T2D patients who treat with metformin against those treated with sulfonylureas showed a decreased incidence of hepatocellular carcinoma in metformin-treated patients (56% risk reduction) (Murff *et al.*, 2018).
A cohort study involving 1,460 patients with liver cancer demonstrated that metformin reduced the mortality rate by 53%, where metformin exposure at the time of liver cancer diagnosis (Currie *et al.*, 2012).
Different related studies have also shown that the development of liver cancer is reduced by 62% in Type 2 DM patients who receive metformin (Jiralerspong *et al.*, 2009). Furthermore, T2D patients and nondiabetic patients who were exposed to metformin show significantly improved prognosis (five-year survival of 60.5% versus 64.7%) (Chen *et al.*, 2011).

4.6: Other cancers

Survival advantages in diabetic patients treated with metformin have been reported for several other cancer types, for example, in epithelial ovarian cancer metformin showed a significant reduction in cancer stem cells and improved the OS indicator (Brown et al., 2020).
In addition, non-muscle-invasive bladder cancer (NMIBC) reported that using metformin can increase the OS indicator and increase disease-specific survival depending on the accumulation of metformin in the urine prior to excretion (Wang et al., 2022).
Whereas endometrial cancer (EC) studies found mixed results, one study showed no relation between metformin and anti-cancer effect, while another study indicated that metformin can alter the EC receptor signaling thus enhancing the anti-tumor effects. otherwise, a recent meta-analysis in endometrial cancer suggested that metformin does not have an antiproliferative role and can't be used as adjuvant therapy to progesterone in patients seeking to spare their fertility (Petchsila et al., 2020).
In multiple myeloma, metformin indicated a decreased incidence of mortality. also, many studies demonstrated the association between MM and decreasing the developmental risk of monoclonal gammopathy of unknown significance (MGUS) by 1% per year through patients taking metformin. (Boursi et al., 2017).
While in brain tumors (glioma), the specific role of metformin remains lacking in evidence, but available studies have indicated many properties of metformin to be used in the managing of brain tumors. In an animal model study (rat model), metformin was found to be distributed through the central nervous system and can enter the blood-brain barrier (BBB). Moreover, metformin showed reductions in symptoms that accompany brain tumors like vascular brain edema and neuro-symptoms (Seliger et al., 2019).
however, studies regarding other cancers like lung, cervical, gastric, head, and neck have shown the role

of metformin in increasing the RFS, CSS, and OS indicators like in lung cancer increased OS or increased RFS and OS in cervical cancer, or in gastric cancer increased OS and CSS, there is no change in the OS regarding head and neck cancer (Malek et al., 2013) (Figure3).

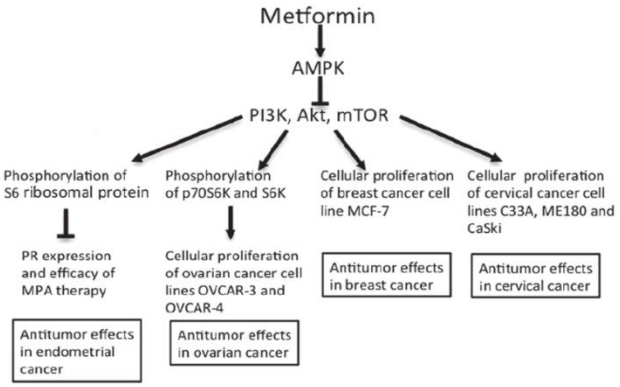

Figure 2: Anti-cancer effect of metformin in various types of cancer (Kouji et al., 2016).

Limitation of metformin evidence in cancer

Generally, Clinical trials face many challenges and limitations, were not all work with animal models either in vitro or in vivo interprets into clinical results in humans. Although, there is much laboratory evidence that appears to livelihood the role of metformin in cancer prevention and treatment, in many instances, the concentrations of metformin used in in vitro and preclinical studies are well higher than expected therapeutic concentrations of plasma reached in humans, and not all cancer types respond to metformin in the same way (Rizos & Elisaf, 2013).

In addition, bias is one of the most common statistical problems faced by observational studies, which results from problems in the study design or unknown and unmeasured factors. however, several bias problems like time-lag, time-related, immortal time, and time-window biases were found in many observational studies conducted to evaluate the association between metformin and the development risk of various cancer types and survival rates.

In conclusion, some observational studies may be inadequate to reveal significant associations and others may insufficiently deal with varying changes like insulin resistance grading, and differences in disease severity (Suissa & Azoulay, 2012).

4. Conclusion

Metformin a small orally molecule drug has constantly shown its anti-tumor effects through several observational and clinical trials. additionally, it has indicated a reduced risk of cancer and incidence of death rates among individuals receiving metformin. However, these results may help in interpreting to benefits of experimental tests, and several hypotheses that can guide future studies.

The anti-tumor identifying mechanisms of metformin faced numerous limitations that affect their interpretation in the clinic, for example, some authors have indicated that the metformin concentrations used in animal models and clinical studies were pointedly higher than expected therapeutic concentrations in humans. Moreover, there is an urgent need to improve the immunological, microbiological environments, and in vivo stem cell cancer models to review tumor heterogeneity and predict better clinical results.

Notably, several studies were conducted to demonstrate the anti-tumor role of metformin either employed a few cases or patients with late cancer stages and both confused the results.

In conclusion, to optimize the antitumor efficacy of clinical studies related to metformin there are critical requirements to carry out further research interesting how to improve the molecular and pharmaceutical structure and evaluate the role of metformin in combination with other drugs.

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