

**Review Article:****Metformin and lung cancer****Sai Yendamuri***Department of Thoracic Surgery, Roswell Park Cancer Institute, Buffalo, New York***ABSTRACT**

Metformin, a commonly used anti-diabetic drug has emerged as a potential anti-cancer drug. Over the last few years, a mass of epidemiologic, outcomes and preclinical data has emerged demonstrating the potential clinical relevance and the mechanistic basis of the anti-cancer activity of this well tolerated drug in lung cancer. This review article summarizes this evidence that supports the trial of this drug in lung cancer.

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**INTRODUCTION**

Lung cancer is the most common cause of cancer related mortality in the United States. Diabetes is a common co-morbidity in lung cancer patients. Metformin is one of the first choices as an oral anti-diabetic agent and, therefore, the intersection of metformin therapy and the lung cancer state is substantial. Over the last few years, several reports examining the association of metformin with lung cancer have been published, including evidence from epidemiological studies, outcome studies, in-vitro and in-vivo data and mechanistic studies (*vide infra*). This paper summarizes this existing data and attempts to synthesize several lines of evidence into a coherent argument for the use of metformin as an anti-cancer agent in lung cancer.

**Metformin and lung cancer incidence**

A recent comprehensive meta-analysis evaluated 24 of the 412 studies reviewed. An examination of the risk of incident cancers patients with diabetes mellitus across these 24 studies demonstrated a decreased risk of incident cancers for colorectal, hepatocellular and lung cancer [ relative risk (RR) 0.67, 95%

confidence intervals (CI) = 0.45-0.99]. The results were driven mostly by retrospective studies. In this meta-analysis,<sup>1</sup> only few studies<sup>2,3</sup> contained lung cancer data. This represents a decrease of risk by nearly 33% in the incidence of lung cancer. A similar study conducted in Taiwan<sup>4</sup> demonstrated no increased risk in the incidence of lung cancer in patients with diabetes mellitus, but did demonstrate a statistically significant decrease in its incidence with the use of metformin. This analysis of drug usage was restricted to *patients with diabetes only*. Of interest, use of thiazolidinediones as well as alpha-glucosidase inhibitors seems to have a beneficial effect as well. In contrast, the use of sulphonylureas had a statistically insignificant increase in the incidence of lung cancers.

The hypothesis that the administration of metformin can prevent the causation of carcinogen induced lung cancer was tested in an animal model.<sup>5</sup> The investigators used the known development of Kirsten rat sarcoma viral oncogene homologue (K-ras) mediated lung tumours in A/J mice exposed to the tobacco specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) to

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compare the effectiveness of metformin as a chemopreventive agent. In this study,<sup>5</sup> the investigators used oral metformin to achieve plasma metformin levels (0.5-2 µg/ mL) comparable to human beings. Metformin was administered for 13 weeks starting 1 week after exposure to NNK. Overall tumour burden was reduced by 39% at a dose of 1 mg/mL and to 53% at 5 mg/mL. At this dose the mechanistic target of rapamycin (mTOR) pathway did not seem to be inhibited. Intraperitoneal dosing of metformin did increase plasma levels and this decreased the tumour burden by 72%. At this dose, the mTOR pathway was inhibited. This model clearly shows that metformin prevented tobacco carcinogen-induced tumour growth in a non-diabetic mouse model of lung tumorigenesis, probably through multiple mechanisms.

### **Metformin and outcomes of patients with lung cancer**

Following the lead of epidemiologic data published, investigators have examined the association between lung cancer outcome and exposure to metformin. In one study,<sup>6</sup> the outcomes of more than 4000 patients with advanced lung cancer treated in five hospitals over a period of 5 years (2004 – 2009) were examined. Of these, 99 patients with diabetes were selected after exclusion of patients with brain metastases, those receiving radiation therapy, patients receiving both metformin and insulin and patients receiving adjuvant chemotherapy less than 12 months prior. Compared to patients treated with insulin or with other drugs, patients treated with metformin had an improved progression free survival (4.7 months vs. 6.7 months vs. 8.4 months;  $p=0.002$ ) and overall survival (13.1 months vs. 13.0 months vs. 20 months;  $p=0.007$ ). A population- based study<sup>7</sup> examined the outcomes of 112,408 individuals with cancer; 7.5% of these patients had diabetes. Compared to patients without diabetes, those

with diabetes had a higher cancer-related mortality if afflicted with breast or prostate cancer, but a lower cancer-related mortality when afflicted with lung cancer [hazard ratio (HR) 0.84; 95% CI= 0.77-0.92]. When the influence of anti-diabetic medication was examined, patients treated with metformin had a better overall cancer mortality than non-diabetic patients and those treated with sulphonylureas and insulin did worse. Site-specific analyses of metformin usage revealed no benefit of metformin usage in lung cancer if used before cancer diagnosis. However, in those used 3 months or less after cancer diagnosis a beneficial effect was seen (HR 0.767; 95% CI= 0.59-0.997).

### **Mechanism of action of metformin**

The primary mechanism of action of metformin that has been studied is in its role as an antidiabetic drug. In this context, the antidiabetic activity has been explained as being due to suppression of hepatic gluconeogenesis and increase in peripheral glucose uptake, possibly by upregulation of the facilitated glucose transporter, member 4 (GLUT-4) protein transporter. In addition, cellular metabolism is altered towards increased glycolytic activity, mimicking stress. This stress leads to a high adenosine monophosphate (AMP) / adenosine triphosphate (ATP) ratio, triggering the activation of adenosine monophosphate activated protein kinase (AMPK) by the liver kinase B1 (LKB1) protein kinase.

Examination of potential mechanisms of action of the anti-cancer effects of metformin has been primarily focused on cancer cell lines. The phenotypic effects of metformin on four lung cancer cell lines, each representing a major histotype: RERF-LC-AI (squamous cell cancer), A549 (adenocarcinoma), IA-5 (large cell carcinoma) and WA-hT (small cell carcinoma) were evaluated in a study.<sup>8</sup> Phenotypic effects were only apparent after prolonged exposure (10 days) to metformin and

not short-term exposure (24 hours). Decreased cell proliferation (trypan blue staining) and clonogenicity (crystal violet staining) were observed on exposure to metformin. Assessment of apoptosis by Hoechst staining and caspase 3, 8 and 9 activity levels did not reveal induction of apoptosis except in WA-hT. Cell cycle analysis demonstrated G0/G1 accumulation in all four cell lines. Interestingly, cisplatin actually reversed the effects of metformin in all cell lines except A549. Therefore, this set of experiments suggests that metformin works by the cell cycle arrest without necessarily inducing apoptosis.

In contrast, another study<sup>9</sup> demonstrated an induction of apoptosis in A549 and H1299 lung cancer cell lines at similar doses of metformin by Annexin V staining. As it is well known that metformin acts via activation of the AMPK pathway<sup>10</sup> which is an upstream regulator of the mitogen activated protein kinases (MAPKs), these pathways were investigated. The investigators demonstrate activation of the JNK/p38 MAPK pathway without an increase in the amount of proteins. Inhibitors of these pathways abrogated this effect significantly. Another mechanism of action explored by the investigators was growth arrest-and deoxyribonucleic acid damage-inducible gene 153 (GADD153) that is upregulated by metabolic stress. Metformin treatment upregulates GADD153 and inhibition of GADD153 decreased the induction of apoptosis. Another molecule important in AMPK activation and energy balance is Caveolin-1 (Cav 1). It has also been demonstrated that Cav1 is required for metformin action in non small-cell lung cancer (NSCLC) cells.<sup>11</sup> In a series of experiments in Calu-1 and Calu-6 cells, the investigators show that Cav-1 silencing reduced the inhibitory effect of metformin on insulin like growth factor-1 (IGF-1) dependent Akt phosphorylation. A third molecule important in this pathway

is carnitine palmitoyltransferase 1C (CPT1C). Another study<sup>12</sup> has shown that CPT1C is an important downstream mediator of the metabolic stress response coordinated by AMPK. Consistent with other data, when CPT1C is silenced, the xenografts thus formed are unresponsive to the effects of metformin, despite AMPK activation.

A consistent observation of the effect of metformin on cancer cells is an arrest at the G1 phase. This observation was followed up by an examination of proteins involved at that checkpoint.<sup>13</sup> In a series of experiments conducted on FaDu and Detroit 562 head and neck squamous cancer cell lines, the investigators show that AMPK activation leads to the inhibition of the global translational machinery by phosphorylation of eukaryotic translation initiation factor binding protein 1 (4E-BP1), a eukaryotic translation initiation factor. This leads to a decrease in the amount of cyclin E, cyclin dependent kinase (Cdk), Cdk4, Cdk6, inhibitor kinase 4A/p16 (INK4A/p16) and cyclin D1, leading to cell cycle arrest. A larger study of this phenomenon compared the inhibition of translation by examining the perturbation of polysome associated messenger ribonucleic acids (mRNAs) after exposure to metformin and mTOR inhibitors.<sup>14</sup> Metformin altered a substantial proportion of polysome associated mRNAs and there was considerable overlap with those altered by mTOR inhibitors, suggesting common pathways influenced by both sets of drugs.

### **Metformin and microRNAs**

A comprehensive analysis of the metabolic pathways induced by metformin has been published.<sup>15</sup> In this study,<sup>15</sup> the authors performed a metabolomic analysis of the effect of metformin on the breast cancer cell line SUM159PT. Both *in-vitro* and *in-vivo* analyses demonstrated a conversion of the metabolic state of the cells from anabolic to a catabolic

state. A reduced utilization of the pentose phosphate pathway, a higher glycerophosphocholine to phosphocholine ratio and an increased utilization of aminoacids for energy production instead of macromolecule synthesis were seen. MicroRNA profiling of metformin treated cells showed an upregulation of microRNAs predicted to be involved in metabolic pathways. This upregulation seems to be mediated by the upregulation of DICER which is in turn due to the increased binding of E2F at the endonuclease in the RNase III family (DICER) promoter. DICER presence was required for the metabolic effects of metformin and DICER overexpression mimicked the metabolic effects of metformin. The investigators further showed that miR-33a upregulation led to downregulation of c-MYC that mediates the metabolic effects of metformin.

The expression of microRNAs in Michigan Cancer Foundation-7 (MCF-7) breast cancer cells treated with metformin to controls were compared in a study<sup>16</sup> and it was found that metformin upregulated the expression of the tumour suppressor gene let-7a an impressive 18 fold. In addition, this study<sup>16</sup> demonstrated that metformin abrogated the induction of miR-181a as well as mammospheres by the epithelial mesenchymal transition (EMT) inducer, transforming growth factor-beta (TGF $\beta$ ), suggesting that one of the mechanisms of action of metformin may be the prevention of an EMT phenotype. Consistent with these results, Bao et al demonstrated that in pancreatic cancer cell lines, metformin decreased the cell proliferation, migration, invasiveness and cancer stem cell (CSC) markers genes such as CD44, epithelial cell adhesion molecule (EpCAM), enhancer of zeste homologue 2 (EZH2), Notch-1, Nanog and octamer binding transcription factor (OCT4). This was associated with re-expression of several microRNAs such as let7a, let7b, miR-200b, miR-200c, miR26a and miR101.<sup>17</sup> Another study<sup>18</sup> demonstrated

similar results in the gastric cancer cell line MKN74 where exposure to metformin for 72 hours demonstrated predominantly upregulation of microRNAs. While the highest up-regulated microRNA in this study was miR-638, there was upregulation of miR-200a, miR200c and members of the let 7 family.<sup>18</sup>

### **Metformin and senescence**

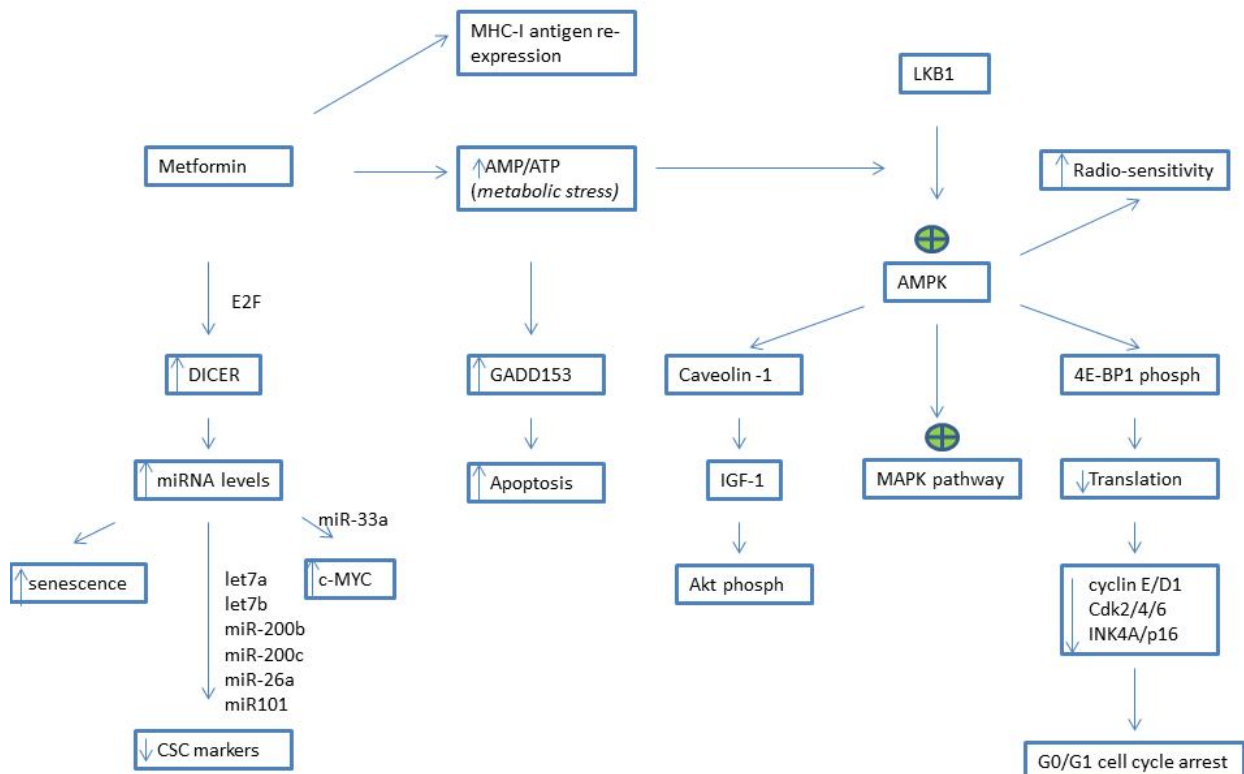
Senescence is thought to be a response that protects against cancer progression. Using senescence prone murine embryonic fibroblasts, Metformin dramatically lowered the threshold for DNA damage inducing agents to induce senescence.<sup>19</sup> The authors<sup>19</sup> also demonstrated that human diploid fibroblasts exposed to metformin chronically developed senescence in an accelerated fashion as evidenced by more than 20-fold induction of members of the miR-200 family. In addition, metformin inhibited the proliferative potential of induced pluripotent cells that are usually resistant to senescence. Therefore, the ability of metformin to induce senescence makes it an attractive potential therapeutic agent for cancer.

### **Other mechanisms**

An alternate mechanism of action of metformin's anti-cancer role was demonstrated where the authors demonstrate that exposure of human epidermal growth factor receptor 2 (HER-2) overexpressing cells to metformin leads to robust re-expression of the major histocompatibility complex-1 (MHC-I) antigen expression, potentially increasing the immunogenicity of tumour cells to the body's innate tumour responses.<sup>20</sup> The multitude of mechanisms involved in the anti-cancer activity of metformin are summarized in Figure 1.

### **Is metformin effective in non-diabetic persons?**

All the above preclinical and mechanistic data has encouraged the exploration of the use of metformin for all cancer patients, not just diabetic cancer patients. This assumption may



**Figure 1:** Schematic depicting the multiple mechanisms demonstrated to be involved in the anti-cancer activity of metformin

↑ = increases; ↓ = decreases; ⊕ denotes activation; CSC = cancer stem cell; MHC=major histocompatibility complex; LKB1=lives kinase B1; AMP=adenosine monophosphate; ATP=adenosine triphosphate; GADD=growth-arrest and deoxyribonucleic acid damage-inducible genes; AMPK=adenosine monophosphate activated protein kinase; IGF=insulin like growth factor

not be valid. Several *in-vivo* experiments demonstrate that the effect of metformin in cancer depends on the diet provided.<sup>21-23</sup> Evidence is available demonstrating that metformin represses tumour growth of Lewis lung cancer cell (LLC1) subcutaneous tumors only in mice treated with a high energy diet that induces hyperinsulinism.<sup>23</sup> These results were validated by another study that demonstrated a similar result in an orthotopic syngeneic model of breast cancer using the triple negative 66c14 tumor cells in Balb/c mice. Interestingly, in this model, while the effect of metformin was seen in the primary tumor, no effect was seen in metastases. A follow up study<sup>22</sup> examined the interaction of the LKB1 gene expression status with diet on the influence of metformin in tumor response. The authors<sup>22</sup> demonstrate that metformin's anti-tumor responses were evident only in the

presence of a hyperinsulinaemic state or in tumors with LKB1 depletion and predict that these may be biomarkers of response to metformin in clinical scenarios. These predictions were borne out somewhat in a recent study,<sup>24</sup> that examined the effect of insulin resistance measured using a homeostasis model assessment (HOMA) index on the effect of metformin on the change in Ki-67 index in breast cancers in non-diabetic patients before and after treatment. Patients with insulin resistance had a decrease in the Ki-67 index whereas patients without insulin resistance had a mean increase in Ki-67 index, suggesting that metformin response may be dependent on the metabolic state of the patient as a whole.

### Metformin and treatment resistance

Radiation therapy is a known inducer of the AMPK pathway and it is reasonable to

hypothesize that metformin treatment may potentiate this effect. This hypothesis was tested using cancer cell lines from lung, prostate and breast cancer.<sup>25</sup> After confirming induction of AMPK in these cell lines with radiation treatment independent of LKB1 status, the investigators demonstrated induction of resistance to ionizing radiation by AMPK inhibitor. They also demonstrated a sensitization to ionizing radiation by metformin as measured by surviving fraction after 2 Gy of exposure, supporting their hypothesis. In a similar study<sup>26</sup> using the breast cancer cell line MCF7 the authors reported that metformin selectively kills cancer stem cells and increases the radiosensitivity of both MCF7 human breast cancer cells as well as FSall mouse fibrosarcoma cells.

Similarly, given the importance of the AMPK pathway in cancer, combination of metformin with chemotherapeutic agents seems attractive. Another study<sup>27</sup> showed that in breast cancer cell lines, metformin potentiated the cytotoxic effects of carboplatin, paclitaxel, doxorubicin and the mTOR inhibitor RAD001. These *in-vitro* effects were further bolstered by *in-vivo* experiments.<sup>28</sup> Using breast cancer, lung cancer (A549) and prostate cancer cell lines, the investigators showed the potentiation of the cytotoxic effects of paclitaxel, carboplatin and doxorubicin in xenografts.<sup>28</sup> In addition, this combinatorial treatment prevents long term relapse suggesting an effect on the cancer stem cell sub-population as well.

Recent epidemiologic and clinical data, thus point out that metformin has emerged as a potential anti-cancer drug. Metformin's anti-cancer effects seems to be mediated by activation of the AMPK pathway, increasing immunogenicity of tumour cells, altering susceptibility of senescence and by killing cancer stem cells. However, enthusiasm for the clinical study of this drug in the general cancer population should be tempered by the

possibility that the anti-cancer effects may be restricted to patients with specific metabolic states, such as diabetics. Further studies are needed to dissect out this association and to further understand the effects of metformin on the tumour microenvironment as well.

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