Review

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Metformin-induced anticancer activities: recent insights

Abstract: Metformin is a widely used antidiabetic drug, and there is evidence among diabetic patients that metformin is a chemopreventive agent against multiple cancers. There is also evidence in human studies that metformin is a cancer chemotherapeutic agent, and several clinical trials that use metformin alone or in combination with other drugs are ongoing. *In vivo* and *in vitro* cancer cell culture studies demonstrate that metformin induces both AMPK-dependent and AMPK-independent genes/pathways that result in inhibition of cancer cell growth and migration and induction of apoptosis. The effects of metformin in cancer cells resemble the patterns observed after treatment with drugs that downregulate specificity protein 1 (Sp1), Sp3 and Sp4 or by knockdown of Sp1, Sp3 and Sp4 by RNA interference. Studies in pancreatic cancer cells clearly demonstrate that metformin decreases expression of Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated genes, demonstrating that one of the underlying mechanisms of action of metformin as an anticancer agent involves targeting of Sp transcription factors. These observations are consistent with metformin-mediated effects on genes/pathways in many other tumor types.

Keywords: chemoprevention; chemotherapeutic; metformin; Sp transcription factors.

Introduction

Metformin or *N,N*-dimethylbiguanide is a low molecular weight (MW: 129) compound that has been used extensively as a constituent of herbal medicines for treatment of multiple ailments (Witters, 2001; Romero et al., 2017). Professor Jean Sterne in France recognized the importance of metformin (or Glugophage) as an antidiabetic agent and championed its clinical applications for decreasing insulin resistance in diabetics in the mid-1970s in Europe (Sterne, 1959). In 1995, metformin was subsequently approved by the US Food and Drug Administration, and metformin is now widely used as a relatively safe, low-cost drug for treating diabetes (Bailey and Turner, 1996; Nathan et al., 2009; Inzucchi et al., 2015). A recent review of metformin by Romero and coworkers (Romero et al., 2017) pointed out that metformin is rapidly gaining status as 'the aspirin of the 21st century', and its use has been associated with improvements in or prevention of many diseases. Figure 1 illustrates some of the health promoting, chemopreventive and chemotherapeutic effects of metformin, which include diabetes, preeclampsia, liver and kidney diseases, cardiovascular diseases, mortality and aging, multiple sclerosis, polycystic ovary disease, modulation of gut microorganisms and metabolites, erectile function and cognitive function (Haffner et al., 2005; Goldberg et al., 2013; Bannister et al., 2014; Cheng et al., 2014; Guo et al., 2014; Bhat et al., 2015; Feng et al., 2015; Goldberg et al., 2015; Victor et al., 2015; Barzilai et al., 2016; Brownfoot et al., 2016; Negrotto et al., 2016; Crowley et al., 2017; Patel et al., 2017; Pollak, 2017; Wu et al., 2017). These impacts on multiple human diseases are also supported by extensive studies on metformin in several model organisms including laboratory animals and suggest that metformin may even surpass aspirin in terms of its broad impact on multiple diseases. There are literally thousands of publications on the mechanisms of action of metformin and the effects of this compound on different pathways. Some of these responses may be due to mitochondrial effects leading to decreased metabolic activity and activation of AMPK and subsequent inhibition of mTOR (Barzilai et al., 2016; Hart et al., 2016; Romero et al., 2017). In this review, we will examine the impacts of metformin on both cancer chemoprevention and chemotherapy and also identify some important underlying pathways associated with these effects.

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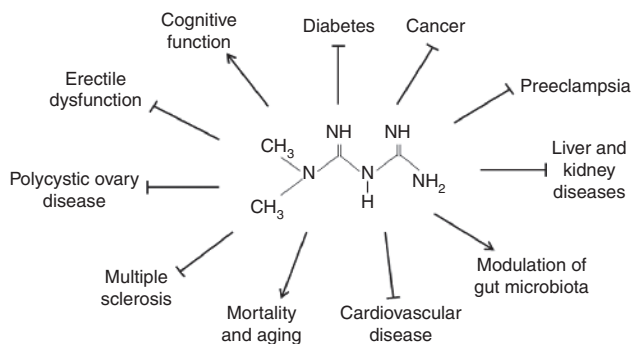
Figure 1: Metformin and diseases.
The antidiabetic drug metformin plays a role in protecting against several adverse health effects in humans (Haffner et al., 2005; Goldberg et al., 2013, 2015; Bannister et al., 2014; Cheng et al., 2014; Guo et al., 2014; Bhat et al., 2015; Feng et al., 2015; Victor et al., 2015; Barzilai et al., 2016; Brownfoot et al., 2016; Negrotto et al., 2016; Crowley et al., 2017; Patel et al., 2017).
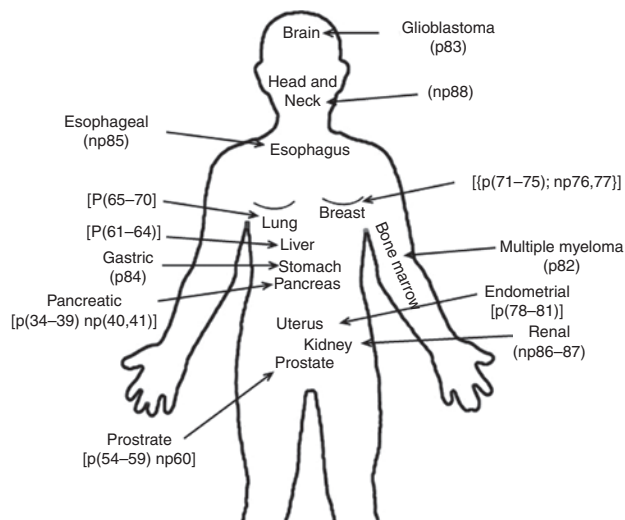

Figure 2: Metformin as a cancer chemopreventive agent.
Metformin inhibits development of several cancers: p, protective; np, not protective (references in brackets).

Metformin and cancer – human studies

Introduction

Examination of PubMed showed that under the keywords 'Metformin' or 'Metformin and Cancer', there were listings for 15 303 and 3493 publications, respectively. Most of the early indications that metformin plays a role in modulating cancer development were observed in papers reporting the incidence of various cancers in diabetics vs. non-diabetics or diabetics treated with other pharmaceuticals. Analysis of individual studies for the chemopreventive effects of metformin demonstrates consistent results for some cancers but highly variable results for patients with other cancers. Several studies have investigated cancer mortalities and sometimes risks in several populations (Gandini et al., 2014; Lega et al., 2014; Wu et al., 2015; Gong et al., 2016; Haukka et al., 2017; Hicks et al., 2017), and this included one report which was a meta-analysis of 265 studies (Wu et al., 2015). The results demonstrate that metformin decreases the overall risk and mortality from cancer, but effects with individual cancers are variable and in some cases there may be an increased benefit for increased use of metformin.

Chemoprevention

The cancer chemopreventive effects of metformin have been extensively investigated (Figure 2) in many types of cancer, including pancreatic cancer patients, in which diabetes is also a risk factor for this cancer (reviewed in Li and Abbruzzese, 2010; Pollak, 2012; De Souza et al., 2016). Early studies in patients from the MD Anderson Cancer Center showed that metformin use in diabetic patients was associated with a decreased risk and decreased mortality from pancreatic cancer (Li et al., 2009; Sadeghi et al., 2012). These results were confirmed in several subsequent studies worldwide showing that in pancreatic patients, metformin use was associated with increased survival, and these observations were strong in patients with resectable pancreatic tumors (Jo et al., 2015; Ambe et al., 2016; Kozak et al., 2016; Lee et al., 2016; Jang et al., 2017). By contrast, it was also reported that there was no association between metformin use and pancreatic cancer survival (Walker et al., 2015; Frouws et al., 2017), suggesting that the benefits of metformin use for pancreatic cancer patients may be only for subsets of patients (e.g. with resectable tumors).

A review summarized several retrospective and case-control studies on metformin and colon cancer, which suggest that metformin decreases the risk and mortality from colon cancer, and improved overall survival was also observed in a meta-analysis of studies (Lee and Kim, 2014; Meng et al., 2017). Moreover, one study also reported that metformin decreased formation of adenomas and polyps in patients after polysectomy, demonstrating the effectiveness of this drug as a chemopreventic agent that decreases formation of precancerous lesions (Higurashi et al., 2016). Most studies show that metformin decreases the risk or mortality of colon cancer patients with diabetes

(Garrett et al., 2012; Lee et al., 2012; Spillane et al., 2013; Fransgaard et al., 2016; He et al., 2016; Paulus et al., 2016; Ramjeesingh et al., 2016; Park et al., 2017), although one report observed protection in female but not male patients (Park et al., 2017). By contrast, a population-based study in Great Britain (Mc Menamin et al., 2016) did not observe a protective effect of metformin and the reason for this outlier report is unclear. A number of studies in diabetics with prostate cancer also showed that metformin decreased the incidence and/or mortality from prostate cancer (Yu et al., 2014; Deng et al., 2015; Hwang et al., 2015; Raval et al., 2015; Stopsack et al., 2016; Wang et al., 2016), whereas prostate cancer chemoprevention was not observed in a patient group from the United Kingdom (Azoulay et al., 2011). Metformin decreased liver cancer risk or mortality in several studies in diabetic patients (Lai et al., 2012; Chen et al., 2013; Singh et al., 2013; Zheng et al., 2013), and similar results were observed for lung cancer patients (Tsai et al., 2014; Kong et al., 2015; Lin et al., 2015; Tian et al., 2016; Wan et al., 2016; Lin et al., 2017).

Metformin also influenced outcomes of diabetic patients with hormone-dependent breast and endometrial cancers. Meta-analysis of several studies showed that metformin decreased mortality but not incidence of breast cancer in diabetics (Yang et al., 2015), and several other studies also reported decreased mortality. However, there were some differences in the chemopreventive effects of metformin that were dependent on tumor classification (El-Benhawy and El-Sheredy, 2014; Xiao et al., 2014; Kim et al., 2015; Vissers et al., 2015). For example, among the Luminal A, Luminal B (high Ki67) and Luminal B (ErbB2^{+}) subtypes of breast cancer, metformin significantly decreased the mortality from breast cancer (Xiao et al., 2014). By contrast, one study showed that metformin did not affect survival of triple negative breast cancer patients (Bayraktar et al., 2012), and another report showed no survival benefits for breast cancer patients using metformin (Oppong et al., 2014). Meta-analysis of endometrial cancer studies reported that metformin decreased mortality (Perez-Lopez et al., 2017), and this was confirmed in other studies (Ko et al., 2014; Nevadunsky et al., 2014; Ezewuiro et al., 2016), including one indicating that diabetic patients only with non-endometrioid endometrial cancer benefitted from metformin (Nevadunsky et al., 2014). Diabetics using metformin compared to other antidiabetic drugs exhibit decreased mortality and/or incidence of multiple myeloma (Wu et al., 2014), glioblastoma (Adeberg et al., 2015) and gastric cancer (Kim et al., 2014), but no significant differences were observed for esophageal cancer (Agrawal et al., 2014), renal (Hakimi

et al., 2013; Psutka et al., 2015) and head and neck cancers (Becker et al., 2014). Thus, it is apparent that metformin is a chemopreventive agent for many but not all cancers, and like many other anticancer agents, metformin is likely to target specific tumor subtypes which, with the exception of breast cancer, have yet to be identified.

Chemotherapy

The chemopreventive effects of metformin have been determined in diabetic patients with cancer, and this may not necessarily be representative of the effects of this compound in non-diabetics. Nevertheless, the exciting effects of metformin as a cancer chemopreventive agent coupled with its effects on cancer cells in culture and animal models have led to the initiation of over 100 clinical trials that are investigating metformin as a chemotherapeutic agent (Romero et al., 2017). Results of some studies evaluating the cancer chemotherapeutic efficacy of metformin are summarized in Table 1, and this includes some diabetic patient groups but focuses on responses after diagnosis (Bensimon et al., 2014; Bhat et al., 2014; Mitsuhashi et al., 2014; Kordes et al., 2015; Sayed et al., 2015; Chae et al., 2016; Chaiteerakij et al., 2016; Han et al., 2016; Reni et al., 2016; Sivalingam et al., 2016). Most studies had some limitations with respect to duration, number of patients and other variabilities (e.g. diabetic vs. non-diabetic); however, studies with metformin alone or in combination with other drugs exhibited minimal benefits of metformin use. There were some positive results observed for cervical and endometrial cancers; however, a more definitive evaluation of metformin use in cancer chemotherapy is premature pending results of the large number of ongoing clinical trials.

Metformin mechanisms of action as an anticancer agent

Introduction

The antidiabetic activities of metformin have been extensively investigated and this involves interactions of this drug with multiple pathways in tissues that contribute to insulin resistance (reviewed in Pernicova and Korbonits, 2014). The positively charged metformin molecule targets the mitochondria and inhibits complex 1 and ATP

Table 1: Metformin as a cancer chemotherapeutic agent.

Tumor site (reference)	Patients	Biomarker/response
Multiple (Chae et al., 2016)	Different patient groups (review)	Multiple
Pancreatic (Kordes et al., 2015)	Double-blind randomized; 6–8 months; gemicitabine/erlotinib ± metformin	No increase in survival
Pancreatic (Chaiteerakij et al., 2016)	Survival of patients (diabetic) after diagnosis ± metformin	Some survival benefit (not significant)
Pancreatic (Reni et al., 2016)	Randomized Phase II trial PEXG (4 drugs) ± metformin	No significant survival benefit
Prostate (Bensimon et al., 2014)	Multiple cohorts (diabetic) followed after diagnosis ± metformin (3.7 year)	No effects on mortality
Liver (Bhat et al., 2014)	Retrospective (diabetic) study with liver cancer followed after diagnosis	No survival advantage
Lung (Sayed et al., 2015)	Stage 4 NSCLC treated with drug cocktail ± metformin	No survival advantage
Cervical (Han et al., 2016)	Diabetic patients ± metformin; monitored after diagnosis	Significant decrease in mortality
Endometrial (Sivalingam et al., 2016)	Studies including women with endometrial cancer and atypical hyperplasia; short duration ± metformin	Decreased Ki-67
Endometrial (Mitsuhashi et al., 2014)	Preoperative prospective trial (4–6 week)	Decreased Ki-67

formation and this can affect many downstream pathways in multiple tissues and organs (Pernicova and Korbonits, 2014; Romero et al., 2017). It was suggested that the loss of ATP contributes to the inhibition of hepatic gluconeogenesis, and this response is AMPK and LKB1 independent (Foretz et al., 2010). Another study showed that metformin non-competitively inhibits the redox shuttle enzyme glycerophosphate dehydrogenase, and this also results in decreased hepatic gluconeogenesis, which contributes to the antidiabetic activity of metformin (Madiraju et al., 2014). Glucagon-induced gluconeogenesis can also be inhibited by metformin through inhibiting production of cAMP and PKA activity. These and many other mechanisms have been proposed to explain the mechanism of action of metformin as an antidiabetic drug, and it is likely that some of these pathways may contribute to the cancer chemopreventive effects of metformin. However, the mechanisms of the chemotherapeutic effects of metformin may be different, and it is evident from a large number of studies that the effects of metformin are diverse and are tumor and cell type dependent. The future use of metformin alone or in combined therapies requires insight into the various pathways and/or genes affected by metformin in order to design drug combinations that facilitate and optimize drug-drug interactions.

Metformin subcellular targets

The biological effects of drugs depend on drug interactions with intracellular targets, which subsequently lead to a cascade of downstream events that alters a response. Table 2 summarizes several studies that show direct metformin interactions with intracellular targets that

subsequently can contribute to the reported antidiabetic and anticancer activities of this drug, and some of these interactions may be directly associated with the anticancer activities of this drug. For example, metformin activates AMPK in rat hepatoma H4IIE cells, resulting in decreased pS6 phosphorylation, and these responses are abrogated after cotreatment with triethylene tetramine (trien), a copper chelating agent (Logie et al., 2012). This paper does not identify the direct protein(s) target(s) of metformin but indicates that metformin's copper binding properties may contribute to AMPK activation. *In vitro* studies showed that metformin directly inhibited AMP deaminase, resulting in increased AMP levels and activation of AMPK (Ouyang et al., 2011). There is also evidence that inhibitors of mitochondrial complex 1 of the respiratory change can enhance AMP levels and activation of AMPK (Buzzai et al., 2007; Hawley et al., 2016), resulting in inhibition of mTOR and downstream pro-survival signaling pathways. The importance of mitochondrial targeting of metformin was confirmed in studies with metformin coupled with triphenylphosphonium (MitoMet), which enhanced mitochondrial targeting. These mitochondrial-targeted metformin analogs were orders of magnitude more potent than metformin as complex 1 inhibitors, inhibitors of pancreatic cancer cell growth and activators of AMPK and inhibition of mTOR (Boukalova et al., 2016). These results strongly implicate that the anticarcinogenic activity of metformin, is due, in part to inhibition of complex 1. An example of an extramitochondrial activation of AMPK by metformin involves mislocation of mutation K-ras to the cytosol in cancer cell (Iglesias et al., 2013). Metformin displaces active K-ras from the cell membrane via a PKC-dependent pathway; however, evidence for direct metformin-K-ras interactions was not determined. Finally, there is also

Table 2: Direct intracellular targets of metformin.

Target	Implications	Reference
High mobility group box 1 (HMGB1)	Binds to metformin and inhibits its pro-inflammatory activity	(Horiuchi et al., 2017)
Copper interactions	Extensive π-bond interaction with copper; copper competitor blocks metformin-induced pAMPK (activation) and other responses in liver cancer cells	(Logie et al., 2012)
Inhibits PP2A signaling	Inhibits interactions of PP2A subunits and PP2A activity and thereby blocks downstream signaling (murine neurons)	(Kickstein et al., 2010)
Interacts with organic ion transporters	Binds Oct1 and other ion transporters to facilitate uptake and secretion of metformin	(Becker et al., 2009; Tzvetkov et al., 2009; Chen et al., 2010; Segal et al., 2011; Nakamichi et al., 2013; Liang et al., 2015; Yee et al., 2015)
Inhibition of glycerophosphate dehydrogenase	Inhibit mitochondrial GPD (*in vivo*) and thereby blocks gluconeogenesis	(Madiraju et al., 2014)
Inhibition of AMP deaminase activity	This inhibitory effect stimulates glucose transport in L6 cells	(Ouyang et al., 2011)
Decrease K-ras activity	Displaces constitutively active K-ras from cell membrane and inhibits Ras signaling	(Iglesias et al., 2013)
Inhibition of mitochondrial complex 1	Inhibition of complex 1 can lead to multiple effects including AMPK activation	(El-Mir et al., 2000; Owen et al., 2000; Brunmair et al., 2004; Hinke et al., 2007; Wheaton et al., 2014; Boukalova et al., 2016; Hawley et al., 2016)

evidence in neuronal cells that metformin associates with and disrupts the PP2A complex (Kickstein et al., 2010), and it is possible that inhibition of PP2A-dependent phosphatase activity could contribute to the anticancer activity of metformin.

Metformin-mediated anticancer pathways and/or genes in cancer cells

There is strong evidence that metformin inhibits cancer cell proliferation, migration and invasion and induces apoptosis; however, the genes and/or pathways involved are highly variable and differ between cells derived from the same tumor and different tumor type. It is likely that some of this variability between studies may not be due to major mechanistic differences but to the focus of various laboratories on different genes and pathways that may be complementary. As indicated in Table 2, metformin activates AMPK to inhibit mTOR and downstream signaling, and metformin-induced activation of this pathway has been reported in many different cancer cell lines (Buzzai et al., 2007; Zakikhani et al., 2008; Vazquez-Martin et al., 2009; Algire et al., 2010; Ben Sahra et al., 2010, 2011; Kim et al., 2011; Blandino et al., 2012; Cerezo et al., 2013; Storozhuk et al., 2013; Zheng et al., 2013; Takayama et al., 2014; Gollavilli et al., 2015; Yi et al., 2017; Zhang et al., 2017). These represent only a small number of the studies showing that metformin activates AMPK in cancer cell lines; however, due to the tumor-type, genotypic variability and different experimental approaches, the effects of metformin appear highly variable. For example, in breast cancer cell lines in which the effects of metformin are, in part, AMPK dependent, the pathways that are described are highly variable. In one study in SUM159PT breast cancer cells, metformin-induced AMPK-dependent downregulation of Myc, and this was accompanied by the upregulation of microRNA-33a (miR-33a) and Dicer, a key enzyme in formation of miRs (Blandino et al., 2012). It was also reported that metformin inhibited breast tumor and cancer cell growth via upregulating tumor suppressor-like miR-200c and downregulating Myc and Akt2 expression, and this was probably due to AMPK activation but was not determined (Zhang et al., 2017). Another study in ER-negative MDA-MB-231 and BT-549 breast cancer cells showed that metformin activation of AMPK resulted in growth arrest and inhibition of migration and invasion which was linked to induction of G3K3β and SIRT1 and downregulation of Myc and the Myc-regulated oncogenic protein metadherin (Gollavilli et al., 2015). By contrast, several studies show that the anticancer activities of metformin in breast and other cancer cell lines are AMPK independent. For example, metformin directly suppressed expression of the ErbB2/HER2 oncogene in ErbB2-breast cancer cells, and this effect was AMPK independent but dependent on inhibition of the p70S6K1 kinase (mTOR downstream kinase) (Vazquez-Martin et al., 2009). Metformin also increases REDD1 expression (p53 dependent) in prostate,

breast and lung cancer (Ben Sahra et al., 2011), and under glucose-deprived conditions, metformin downregulated p63 in head and neck cancer cells (Yi et al., 2017) and all of these responses were AMPK independent. Thus, the effects of metformin in cancer cells appear to be highly variable.

Mechanisms of action of metformin: are Sp transcription factors a target?

The actions of metformin in cancer cell lines derived from multiple tumors show that this compound decreases cancer cell proliferation, migration and survival, and activation of AMPK is a trigger for inducing many of these responses. Pancreatic cancer was one of the first cancers where it was shown that metformin exhibited chemopreventive activity among diabetics; however, continuing studies gave mixed results (Table 1). Metformin and related biquanides are highly effective anticancer agents in pancreatic cancer cells (Kisfalvi et al., 2009; Rozengurt et al., 2010; Kim et al., 2011; Bao et al., 2012; Gou et al., 2013; Karnevi et al., 2013; Lonardo et al., 2013; Sinnett-Smith et al., 2013; Soares et al., 2013; Li et al., 2017; Rajeshkumar et al., 2017). A recent study showed that metformin suppressed cancer initiation and progression in the LS-Kras$^{G12D/+}$; Trp53$^{fl/+}$; Pdx-Cre(KPC) mouse that expresses an active Ras (Kras$^{G12D/+}$) and mutated p53 in the pancreas and spontaneously develops pancreatic tumors similar to the pancreatic ductal adenocarcinomas (PDACs) observed in humans (Chen et al., 2017). Most *in vitro* studies on metformin in pancreatic cancer cells showed the importance of AMPK activation, the insulin growth factor receptor 1 (IGF-R1), modulation of microRNA (miR) expression and inhibitory effects on pancreatic cancer stem cells.

Studies in this laboratory have focused on the pro-oncogenic activity of specificity protein 1 (Sp1), Sp3 and Sp4 transcription factors (TFs), which are highly expressed in pancreatic and other cancer cell lines, and there are a number of anticancer drugs that act, in part, by targeting Sp transcription factors (TFs) (Wei et al., 2004; Safe and Abdelrahim, 2005; Abdelrahim et al., 2006; Chintharlapalli et al., 2007; Chadalapaka et al., 2008, 2012; Jutooru et al., 2010a,b,c, 2014; Basha et al., 2011; Chintharlapalli et al., 2011; Pathi et al., 2011a,b, 2012, 2014; Chadalapaka et al., 2012; Gandhy et al., 2012; Liu et al., 2012; Sreevalsan and Safe, 2013; Hedrick et al., 2015, 2017; Kasiappan et al., 2016; Safe and Kasiappan, 2016; Jin et al., 2017; Karki et al., 2017; Taoka et al., 2017). Compounds that induce downregulation of Sp1, Sp3 and Sp4 include NSAIDs

such as tolfenamic acid, diclofenac, aspirin and GT-094, a nitroaspirin analog and arsenic trioxide; and natural product-derived anticancer agents such as betulinic acid, curcumin, piperlongumine, cannabinoids, celastrol, phenethylisothiocyanate (PEITC), benzylisothiocyanate (BITC) and the synthetic triterpenoids methyl 2-cyano-3,12-dioxo-oleana-1,9-dien-28-oate (CDDO-Me, bardoxolone) and methyl 2-cyano-3,11-dioxo-18β-olean-1,13-dien30-oate (CDODA-Me) (Wei et al., 2004; Safe and Abdelrahim, 2005; Abdelrahim et al., 2006; Chintharlapalli et al., 2007, 2011; Chadalapaka et al., 2008, 2012, 2013; Jutooru et al., 2010a,b,c, 2014; Basha et al., 2011; Gandhy et al., 2012; Liu et al., 2012; Pathi et al., 2012, 2014, 2011a,b; Sreevalsan and Safe, 2013; Hedrick et al., 2015, 2017; Kasiappan et al., 2016; Safe and Kasiappan, 2016; Jin et al., 2017; Karki et al., 2017; Taoka et al., 2017). The potent anticancer activity of drugs that target downregulation of Sp TFs is due to the subsequent downregulation of pro-oncogenic Sp-regulated genes that contribute to cancer cell growth, survival and migration/invasion. Based on RNA interference (RNAi) experiments, Sp TFs regulate bcl-2, survivin, cyclin D1, vascular endothelial growth factor (VEGF) and its receptors VEGFR1 and VEGFR2, hepatocyte growth factor receptor (c-MET), platelet-derived growth factor receptor 1 (PDGFR), epidermal growth factor receptor (EGFR), ErbB2 (via YY1), insulin-like growth factor receptor (IGFR-1), p65 (NF-κB), cMyc, EZH2, MDR1 and multiple integrins (Wei et al., 2004; Safe and Abdelrahim, 2005; Abdelrahim et al., 2006; Chintharlapalli et al., 2007, 2011; Chadalapaka et al., 2008, 2012, 2013; Jutooru et al., 2010a,b,c, 2014; Basha et al., 2011; Pathi et al., 2011a,b, 2012, 2014; Gandhy et al., 2012; Liu et al., 2012; Sreevalsan and Safe, 2013; Hedrick et al., 2015, 2016, 2017; Kasiappan et al., 2016; Safe and Kasiappan, 2016; Jin et al., 2017; Karki et al., 2017; Taoka et al., 2017). Sp-regulation of some of these genes is cell context dependent, and many are coregulated by Sp and other TFs. Interestingly, inspection of published results for metformin shows that like metformin, knockdown of Sp1, Sp3 and Sp4 also inhibits cancer cell growth and migration and induces apoptosis. In addition, metformin also decreases expression of several Sp-regulated genes, including VEGF (Li et al., 2017), ErbB2 (Soares et al., 2013) and EZH2 (Bao et al., 2012) in pancreatic cancer cells; MDR1, c-Myc, p65 and bcl2 in breast cancer cells (Kim et al., 2011; Zhang et al., 2017); MMP9 in melanoma cells (Cerezo et al., 2013); and also ErbB2 in breast cancer cells (Vazquez-Martin et al., 2009).

The effects of metformin on expression of several putative Sp-regulated genes suggest that Sp1, Sp3 and Sp4 may be targets of metformin, and this was further investigated in Panc1, Panc28 and L3.6pL pancreatic cancer cells.

Metformin (0–20 μm) inhibited growth, induced apoptosis and decreased expression of Sp1, Sp3 and Sp4 in all three cell lines, and these effects suggest that Sp downregulation contributes to the anticancer activity of metformin because Sp knockdown (individually and combined) also inhibits growth and induces apoptosis in pancreatic cancer cells (Nair et al., 2013, 2014).

A major mechanism of action of metformin in both non-cancer and cancer cells is linked to activation of AMPK, which in turn can affect multiple downstream pathways including inhibition of mTOR and induction of autophagy. Interestingly, the induction of autophagy by 2-deoxyglucose, which inhibits glucose metabolism, is significantly attenuated by cotreatment with metformin, and this is accompanied by enhanced p53-dependent apoptosis in prostate cancer cells (Ben Sahra et al., 2010). In addition, the inhibition of mTOR in pancreatic cancer was also linked to the downregulation of Sp1, Sp3 and Sp4 in pancreatic cancer cells (Nair et al., 2014). In these cells, metformin downregulated insulin-like growth factor receptor and decreased Akt phosphorylation in pancreatic cancer cells, and this resulted in decreased mTOR phosphorylation. Knockdown of Sp1, Sp3 and Sp4 by RNAi gave similar results, thus linking metformin-mediated inhibition of mTOR to Sp downregulation by this drug. The effects of metformin as an activator of AMPK were not determined in this study (Nair et al., 2014), but it is possible that activation of AMPK by metformin was also a contributing pathway.

Although a role for AMPK activation by metformin was not determined, we observed that metformin inhibited activation of pmTOR, and mTOR activation was also inhibited after knockdown of Sp1, Sp3 and Sp4 by RNAi (Nair et al., 2014). In addition, metformin also decreased expression of several Sp-regulated genes, including bcl-2, survivin, cyclin D1, VEGF, VEGFR1, fatty acid synthase (FAS), activation of sterol regulatory element binding protein 1 (SREBP) and EGFR (Nair et al., 2013, 2014). Metformin also modulated expression of genes directly linked to mechanistic pathways, and these include decreased Ras activity, repression of miR-27a and induction of miR-regulated ZBTB10 (a transcriptional repressor) and the dual phosphatases mitogen-activated protein kinase phosphatase 1 (MKP1 and MKP5) (Nair et al., 2013, 2014) (Figure 3).

The mechanisms of drug-induced downregulation of Sp TFs are both drug and cell context dependent, and for Panc28 and L3.6pL cells, metformin-mediated Sp downregulation could be blocked by the proteasome inhibitor gliotoxin (Nair et al., 2013). A previous study showed that the NSAID tolfenamic acid also induced proteasome-dependent degradation of Sp1, Sp3 and Sp4 in pancreatic cancer
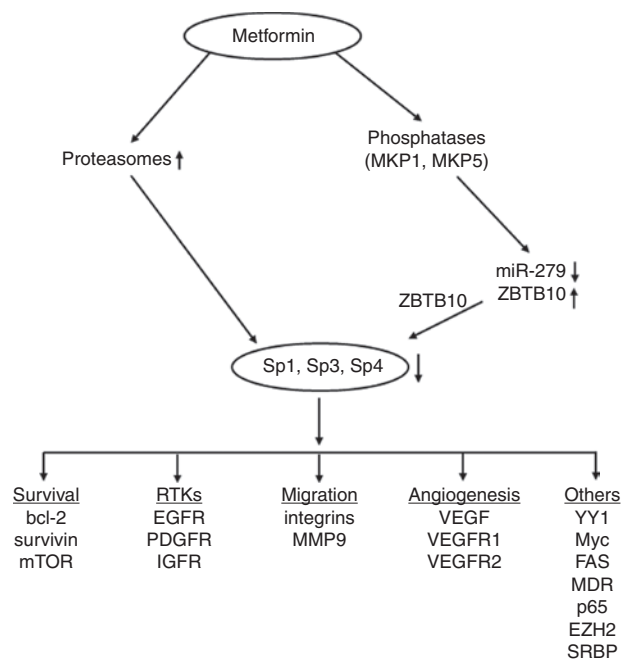

Figure 3: Metformin and Sp transcription factors – a possible underlying mechanism of action.
Metformin downregulates Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated genes in pancreatic cancer cells (Nair et al., 2013, 2014).

cells (Abdelrahim et al., 2006). The mechanism associated with proteasome activation by metformin in pancreatic cancer cells was not determined; however, a recent study reported that metformin-induced proteasome-dependent degradation of the Sp-regulated gene cyclin D1 in ovarian cancer cells, and this was linked to activation of AMPK and GSK3β (Gwak et al., 2017). Thus, the metformin-AMPK-GSKβ pathway may also be responsible for Sp downregulation in pancreatic cancer cells, and there are other reports showing that GSK3β plays a role in proteasome activation (Ichikawa et al., 2015; Wakatsuki et al., 2017). Metformin also downregulated Sp1, Sp3, Sp4 and Sp-regulated genes in the highly invasive Panc1 pancreatic cancer cell line (Nair et al., 2014); however, this response was independent of proteasome activation or induction of ROS, which is a major pathway for degradation of Sp TFs (Safe and Kasiappan, 2016). Metformin-induced Sp downregulation was accompanied by downregulation of miR-27a and induction of the miR-regulated transcriptional repressor ZBTB10, which competitively binds GC-rich Sp binding sites to displace Sp TFs. Metformin-mediated downregulation of miR-27a and induction of ZBTB10 is sufficient to decrease expression of Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated genes (Mertens-Talcott et al., 2007), and we observed that this pathway was inhibited after cotreatment with the phosphatase inhibitor sodium orthovanadate

(SOV) (Nair et al., 2013). There was previous evidence that both mitogen-activated protein kinase phosphatase 1 (MPK1) and MKP5 are induced by compounds (e.g. curcumin and rosiglitazone) (Nonn et al., 2007; Jan et al., 2009; Chen et al., 2011) that also decrease Sp TFs (Jutooru et al., 2010a; Gandhy et al., 2012), and we observed that metformin also induced MKP1 and MKP5 (Nair et al., 2013). Moreover, MKP1/MKP5 overexpression also downregulated miR-27a, induced ZBTB10 and decreased expression of Sp1, Sp3 and Sp4, demonstrating that induction of these phosphatases by metformin was critical for Sp downregulation in Panc28 and L3.6pL cells (Nair et al., 2013). There is also evidence that AMPK activates phosphatases (Berasi et al., 2006; Kim et al., 2010); however, the metformin-AMPK-MKP1/MKP5 pathway was not further investigated in pancreatic cancer cells, and the precise role of AMPK (upstream or downstream) in Sp downregulation has not been determined.

Summary

Metformin exhibits both cancer chemopreventive and chemotherapeutic activity and clearly warrants thorough evaluation for its potential as a single agent or a component of drug cocktails used for cancer treatment. The design of effective drug combinations requires an in-depth knowledge of the underlying mechanisms of action of metformin, and results from our laboratory suggest that many of the effects observed for metformin in pancreatic cancer and possibly other cancers may be due to targeting downregulation of Sp TFs and pro-oncogenic Sp-regulated genes.

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