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Review

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Metformin: On ongoing journey across bladder cancer

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Abstract

Bladder cancer represents the fourth most common cancer in men and ninth most common cancer in women. It is the second most prevalent cancer in men 60 years of age or older in United States. Looking further down, continuing advancements in cancer research could potentially offer more choices for clinician and patient with longer survival and better quality of life. In spite of, bladder cancer represents an ideal tumor model to test and apply cancer prevention strategies; there are limited studies about application of metformin in the management of bladder cancer. Here, I will shed light on the proposed mechanisms of anti-carcinogenic effects of metformin and cohort of these mechanisms with the novel application of metformin as therapy of bladder cancer.

Keywords: metformin; anti-carcinogenic effects; bladder cancer

Introduction

Metformin, a biguanide, was approved by the United States Food and Drug Administration in 1995 as an oral hypoglycemic agent. Given alone or in combination with a sulfonylurea, metformin improves glycemic control and lipid concentrations in patients who respond poorly to dietary control or to a sulfonylurea alone.

In this review, the proposed mechanisms of anti-carcinogenic effects of metformin and correlation of these mechanisms are discussed with bladder cancer according to recent published literatures.

The potential beneficial effects of metformin against cancer are believed to be mediated mainly by one or more mechanisms that I will discuss further: (a) metformin induces growth inhibition, (b) metformin regulates insulin and glucose levels, (c) metformin induce cell death, (d) metformin potentiates the cytotoxicity of chemotherapeutic drugs, (e) metformin's association with oxidative stress, DNA damage and DNA damage response (DDR), (f) immune and hypothalamic effects of metformin and (g) autophagy effects of metformin.

Anti-carcinogenic effects of metformin

Various epidemiologic studies have shown that metformin is associated with reduced risk of cancer in diabetic patients [1, 2]. In addition, several studies have shown that the use of metformin significantly reduces the risk of cancers like breast [3], pancreatic [4] and prostate [5]. Interestingly, a recent meta-analyses/systematic reviews have examined the overall incidence of cancer in patients with diabetes taking metformin versus not and this study concluded that patients with diabetes who are treated with metformin have an approximately one-third reduction in the overall incidence of cancer [6]. Similarly, metformin has also been associated with lower overall cancer-related mortality [7]. Furthermore, the potential antitumor effects of metformin have been evaluated in numerous *in vitro* and *in vitro* studies on several cancer models including breast, endometrial, ovarian, pancreatic, lung, prostate, head and neck carcinomas, acute myeloid leukemia and glioma [8].

Metformin and bladder cancer

Study by Tseng, reported that metformin use is associated with a decreased risk of bladder cancer in Taiwanese patients with type-2 diabetic mellitus [9]. Moreover, a single-institution retrospective cohort study performed from January 1997 to June 2013 to examine the association between metformin use and oncologic outcomes in 421 of diabetic patients undergoing radical cystectomy for bladder cancer and the authors concluded that metformin improves recurrence-free survival and bladder cancer-specific survival in diabetic patients undergoing radical cystectomy [10]. Comparably, retrospective study conducted on 1117 patients with non-muscle-invasive

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bladder cancer (NMIBC) at four institutions between 1996 and 2007 confirmed that patients with diabetic mellitus and NMIBC who do not take metformin seem to be at an increased risk of disease recurrence and progression [11]. Interestingly, a recent study demonstrated that metformin inhibits the proliferation of bladder cancer cells *in vitro* and *in vivo* [12]. The ongoing and upcoming clinical trial with metformin in bladder cancer prevention is given in Table 1.

Table 1 The ongoing and upcoming clinical trial with metformin in bladder cancer prevention.

Trial	*Clinical trial, gov. identifier*	*phase*	*Measured endpoints*
Metformin & simvastatin use in invasive bladder cancer	NCT02360618 observed in tumor cells	Phase II	Level of apoptosis

Proposed mechanisms of anticarcinogenic effects of metformin

Metformin induces growth inhibition

When metformin is transported into the cells, it inhibits mitochondrial complex I (NADH: ubiquinone oxidoreductase) which consider as the first and largest enzyme of the respiratory chain and has a central role in cellular energy production through the coupling of NADH: ubiquinone electron transfer to proton translocation [13]. Thus, metformin has ability to decrease ATP synthesis [14]. As a result, the AMP: ATP ratio in the cell is increased, leading to energy stress and activation of AMPK (AMP-activated protein kinase) [15]. Activation of AMPK leads to a cascade of downstream events resulting in mammalian target of rapamycin (mTOR pathway) down-regulation, which eventually induces protein synthesis arrest and growth inhibition [16, 17]. There are two different multiprotein complexes for mTOR, TORC1 and TORC2, which regulate protein synthesis necessary for cell growth, proliferation, angiogenesis, and other cellular endpoints [18]. Interestingly, mammalian target of rapamycin a member of the phosphatidylinositol 3-kinase (PI3K) cell survival pathway plays an important role in the regulation of cell growth and proliferation by monitoring nutrient availability, cellular energy levels, oxygen levels and mitogenic signals [19]. Aberrant activation of the PI3K pathway has been widely implicated in many cancers, and increased activity of this pathway is often associated with resistance to cancer therapies [20].

Correlation of this pathway with bladder cancer

Several studies concluded that the AMPK pathway might influence both bladder cancer development and progression. A recent study by Liu et al. [21] reported that Rhodiola rosea extract and salidroside inhibit the mTOR pathway and translational initiation via activation of AMPKα in UMUC-3 bladder cancer cells. In addition, PI3K and mTOR have prognostic/predictive value and represent valuable therapeutic targets in bladder cancer [22]. Metformin inhibits the proliferation of bladder cancer cells *in vitro* and *in vivo* through activation of AMPK and mTOR [12]. Moreover, metformin inhibits the growth of bladder cancer cells via indirect activation of AMPK [23, 24], which in turn suppresses the mTOR/ p70 S6 kinase-1 (S6K1) pathway in 253J and RT4 bladder cancer cell lines.

Metformin regulates insulin and glucose levels

It is widely known that cancer cells express insulin as well as insulin-like growth factor (IGF) receptors (IGF-R) and that, besides its metabolic effect, IGF-R promotes proliferation and metastasis [25]. Cancer cells in particular have a constitutively high glucose uptake, independently of IGF-R activation [26]. However, hyperinsulinemia may promote tumor growth by various indirect mechanisms too, such as proliferation of epithelial tissue, increasing bioavailability of steroid sex hormones and serum levels of IGF, as well as disrupting the homeostasis of adipokines, which are cytokines selectively secreted by adipose tissue and thought to be implicated in cancer pathogenesis [27]. In addition, IGF activation promotes vascular smooth muscle cell proliferation and migration, promoting angiogenesis which could contribute to tumor growth [28].

Metformin activates AMPK which results in inhibition of gluconeogenesis in the liver, reducing insulin and glucose levels and increasing glucose uptake in skeletal muscle (a similar way as in metformin-treated diabetic patients) [29]. Metformin is thought to reduce ligand binding to insulin receptors; thus, metformin can indirectly down-regulate the insulin signaling pathway in tumours [30]. Moreover, metformin was shown to directly inhibit insulin induced malignant as well as benign cell growth in an AMPK/mTOR-dependent manner [31]. Recently, metformin was shown to exert a more direct effect on insulin signaling; by down-regulating a downstream target of the insulin receptors called IRS-1 (Insulin Receptor Substrate-1) [30]. The IRS-1 is a widely expressed protein that, in the presence of insulin, becomes phosphorylated by insulin receptors (or by IGF-1-R) resulting in activation of downstream insulin-associated signalling pathways like PI3K-AKT/Protein Kinase B(PKB) and Ras-MAPK [32].

Correlation of this pathway with bladder cancer

Caloric intake appears to affect tumorigenesis through IGF. Higher caloric intake has been associated with an increased incidence of bladder cancer in American men less than 65 years of age [33]. In addition, the decreased caloric intake in mice slowed the growth of bladder tumors and this effect was reversed by IGF-1 administration [34]. Similarly, study found patients with elevated plasma IGF-1 levels to be three times more likely to develop bladder cancer [35]. Interestingly, study demonstrated that cells of human bladder cancer transfected with hsa-miR-96 inhibitor significantly reduced the growth of bladder cancer cells through reduction of mRNA and protein levels of IRS1 [36].

Metformin induces cell death

Metformin was shown to promote cell death across multiple cell lines through both caspase-dependent and caspase-independent mechanisms [37]. It was shown that metformin decreases the expression of anti-apoptotic proteins B-cell lymphoma- 2 (BCL-2), B-cell lymphoma-extra-large (BCL-xL) and Myeloid cell leukemia-1 (Mcl-1), resulting to induction of the pro-apoptotic proteins, BCL-2-associated X Protein (BAX) and BCL-2-associated death promoter (BAD) which lead to activation of caspases and

apoptosis in ovarian cancer (OC) [38]. Metformin is also induced apoptosis by a caspase-independent mechanism involving the activation of PARP (poly (ADP-ribose) polymerase) which results in nuclear translocation of AIF (apoptosis-inducing factor) that leads to apoptosis [39]. More importantly, AMPK/mTOR-mediated decrease of survivin *in vivo* which contributed in metformin-induced apoptosis of gastric cancer cell [40].

Correlation of this pathway with bladder cancer

A recent study deduced that nortriptyline has antitumor on human and mouse bladder cancer cells through induction of both intrinsic and extrinsic apoptosis. It increases the expression of Fas, FasL, FADD, Bax, Bak, and cleaved forms of caspase-3, caspase-8, caspase-9, and PARP. It also decreases the expression of BCL-2, BCL-xL, BH3 interacting domain death agonist, X-linked inhibitor of apoptosis protein, and survivin [41]. Survivin inhibits apoptosis by blocking activation of effector caspases in both extrinsic and intrinsic pathways of apoptosis. Moreover, survivin has also been indicated as a suitable target for developing specific therapy for local treatment of bladder cancer. Thus, survivin is a potentially significant protein with a crucial role in the diagnosis, prognosis, and treatment of bladder cancer [42].

Metformin potentiates the cytotoxicity of chemotherapeutic drugs

Metformin was shown to potentiate the cytotoxic effects of cisplatin *in vitro* [43] and *in vivo* studies; for instance metformin and cisplatin synergistically reduced size, proliferation and mitotic count of OC tumours in mice [44]. Metformin was shown also to potentiate the cytotoxic effects of carboplatin using OC cell lines and primary cultures from OC patients in advanced stages (III-IV) [45]. Moreover, *in vitro* studies showed that metformin acts synergistically with paclitaxel and potentiates its growth inhibitory effects in endometrial cancer [46]. Similarly, a study showed that the combination of metformin with phenethyl isothiocyanate (PEITC) increases growth inhibition and cytotoxicity in OC cell lines in a synergistic manner [47]. Interestingly, several studies have shown the advantages of combining metformin with standard cytotoxic drugs like cisplatin [48], taxol [49], and doxorubicin [50] or with molecular targeted agents such as gefitinib [51]. In addition, metformin showed a synergic effect when used in combination with 5-fluorouracil, in particular affecting CD133+ colorectal cancer cells viability in diabetic patients [52].

Metformin's association with oxidative stress, DNA damage and DNA damage response (DDR)

There are several mechanisms that get activated after DNA damage which occurred to avoid genomic instability; they are known as DNA damage response (DDR). One of the earliest DDRs is the activation of γH2AX as a result of double-strand breaks (DSB). This response occurs within minutes of the damage, thus making it a useful marker of DNA damage. The description of events involved in this activation in mammalian cells leading to γH2AX [53]. The earliest responding proteins are those of the phosphatidylinositol 3-kinase-like family of kinases (PIKK) including ataxia telangiectasia-mutated (ATM), ATM- and Rad3-related (ATR) and the catalytic subunit of DNA-dependent protein kinase (DNA-PKc) [54].

Metformin exerts a protective effect from DNA damage as confirmed in several studies by reduction of DNA damaged signaling, γH2AX expression, γH2AX foci formation, ATM activation or reactive oxygen species (ROS) levels by inhibition of mitochondrial complex I of the respiratory chain [54, 55]. Furthermore, inhibition of complex I compromises the electron flow in the electron transport chain, leading to reduced production of ROS by complexes I and III (mitochondrial ROS producers) [56].

Correlation of this pathway with bladder cancer

Mechanisms governing treatment-induced DNA damage are both central to and predictive of bladder cancer cell treatment sensitivity and exemplify a link between DNA damage resistance and both treatment response and tumour aggression [57]. On the other hand, study conducted by Camargo et al., indicated that no relationship was observed between the amount of DNA damage and the level of hMLH1 (a gene involved in the mismatch repair pathway) and RASSF1 (a tumor suppressor gene) in bladder cancer cells treated with cisplatin and gemcitabine. They also confirmed other alternative pathways might be involved in cisplatin and gemcitabine genotoxicity in bladder cancer cells [58].

Immune and hypothalamic effects of metformin

The concept of immune-modulating effects of metformin was originally proposed in the 1950s by the Philippine physician Garcia [59]. A recent study suggested that metformin can increase the number memory CD8 T cells in wild type mice, and in consequence significantly improve the efficacy of an experimental anticancer vaccine through increased fatty acid oxidation [60]. In addition, Ropelle et al., have shown that hypothalamic AMPK activation in response to metformin reverses cancer anorexia in tumor bearing rats through inhibiting the production of proinflammatory molecules and controlling neuropeptide expression in the hypothalamus [61].

Correlation of this pathway with bladder cancer

A high fraction of adaptive immune markers CD3 (the whole T cell population) and CD8 (T effector cells) in bladder cancer indicated a poor prognosis, thereby emphasising the important role that Tregs play in the suppression of the anti-tumour immune response [62].

Autophagy effects of metformin

Autophagy is a self-degradation process that is important for balancing sources of energy at critical times in development and in response to nutrient stress. It also plays a housekeeping role in removing misfolded or aggregated proteins, clearing damaged organelles, such as mitochondria, endoplasmic reticulum and peroxisomes, as well as eliminating intracellular pathogens. Thus, it is generally thought of as a survival mechanism, although its deregulation has been linked to non-apoptotic cell death [63].

Metformin can enhance autophagy, as AMPK activation is known to upregulate autophagic activity through direct

phosphorylation of unc-51-like kinase and Beclin 1, key molecules involved in the initiation of autophagy [64]. The activation of AMPK by metformin proposes the possibility that improvement in metabolic profiles by metformin might be related to autophagy induction through AMPK activation. In addition, metformin has been shown to enhance disposal of accumulated autophagic vacuoles in β-cells [65]. It has been reported also to enhance autophagic activity in cardiac tissue by facilitating dissociation of the B-cell lymphoma 2 (BCL-2)-Beclin 1 complex through AMPK activation [66] and ameliorating ultra-structural abnormalities associated with diabetes in an animal model of diabetic cardiomyopathy [67]. Interestingly, a recent study reported amelioration of hepatic steatosis by metformin through autophagy activation via sirtuin 1 pathway rather than AMPK pathway [68]. The sirtuin 1 could influence autophagy directly via its deacetylation of key components of the autophagy induction network, such as the products of autophagy genes (Atg) 5, 7, and 8 [69].

Correlation of this pathway with bladder cancer

A recent study conducted by Takahashi et al., showed that the anti-proliferative effects and apoptosis caused by metformin endometrial cancer cells are partially or completely dependent on autophagy [70]. Similarly, several studies have indicated that Troglitazone affects both cell growth and differentiation progress and has an inhibitory effect on urinary cancer cells by activation of autophagy concurrent with the activation of the AMPK signaling pathway [71]. Moreover, further studies have shown that gartanin treatment of bladder cancer cell lines T24 and RT4 resulted in a marked induction of autophagy and apoptosis [72].

Conclusion

Although, there are currently more than one hundred ongoing or upcoming clinical studies assessing the role of metformin in the therapy cancer, only one ongoing clinical trial assessing the role of metformin in the prevention of bladder cancer. In addition, there are limited studies regarding application of metformin in the therapy of bladder cancer. Hence, this review will open the door towards further researches on this direction to confirm or not novel usage of metformin through its ongoing journey across cancer therapy.

Conflicts of interest

Authors declare no conflicts of interest.

References

[1] Evans JM, Donnelly LA, Emslie-Smith AM, Alessi D, Morris A. Metformin and reduced risk of cancer in diabetic patients. B.M.J. 2005; 330(7503):1304–1305.

[2] Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, et al. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. Diabetes Care. 2009; 32(9):1620–1625.

[3] Bodmer M, Meier C, Krahenbuhl S, Jick SS, Meier CR. Long-term Metformin use is associated with decreased risk of breast cancer. Diabetes Care. 2010; 33(6):1304–1308.

[4] Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. Gastroenterology. 2009; 137(2):482–488.

[5] Wright JL, Stanford JL. Metformin use and prostate cancer in Caucasian men: results from a population-based case-control study. Cancer Causes Control. 2009; 20(9):1617–1622.

[6] Thakkar B, Aronis KN, Vamvini MT, Shields K, Mantzoros CS. Metformin and sulfonylureas in relation to cancer risk in type II diabetes patients: a meta-analysis using primary data of published studies. Metabolism. 2013; 62(7):922–934.

[7] Zhang P, Li H, Tan X, Chen L, Wang S. Association of metformin use with cancer incidence and mortality: a meta-analysis. Cancer Epidemiol. 2013; 37(3):207–218.

[8] Würth R, Barbieri F, Florio T. New molecules and old drugs as emerging approaches to selectively target human glioblastoma cancer stem cells. Bio Med Research Int. 2014; 2014:126586.

[9] Tseng CH. Metformin may reduce bladder cancer risk in Taiwanese patients with type 2 diabetes. Acta Diabetol. 2014; 51(2):295–303.

[10] Nayan M, Bhindi B, Yu JL, Hermanns T, Mohammed A, et al. The effect of metformin on cancer-specific survival outcomes in diabetic patients undergoing radical cystectomy for urothelial carcinoma of the bladder. Urol Oncol. 2015; 33(9):e7–13.

[11] Rieken M, Xylinas E, Kluth L, Crivelli JJ, Chrystal J, et al. Association of diabetes mellitus and metformin use with oncological outcomes of patients with non-muscle-invasive bladder cancer. BJU Int. 2013; 112(8):1105–1112.

[12] Zhang T, Guo P, Zhang Y, Xiong H, Yu X, et al. The antidiabetic drug metformin inhibits the proliferation of bladder cancer cells *in vitro* and *in vivo*. Int J Mol Sci. 2013; 14(12):24603–24618.

[13] Baradaran R, Berrisford JM, Minhas GS, Sazanov LA. Crystal structure of the entire respiratory complex I. Nature. 2013; 494(7438):443–448.

[14] Viollet B, Guigas B, Sanz GN, Leclerc J, Foretz M, et al. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond). 2012; 122(6):253–270.

[15] Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metabol. 2005; 1(1):15–25.

[16] Gotlieb WH, Saumet J, Beauchamp MC, Gu J, Lau S, et al. *In vitro* metformin anti-neoplastic activity in epithelial ovarian cancer. Gynecol Oncol. 2008; 110(2):246–250.

[17] Rattan R, Giri S, Hartmann L, Shridhar V. Metformin attenuates ovarian cancer cell growth in an AMP-kinase dispensable manner. J Cell Mol Med. 2009; 15(1):166–178.

[18] Lee DF, Hung MC. All roads lead to mTOR: integrating inflammation and tumor angiogenesis. Cell Cycle. 2007; 6(24):3011–3014.

[19] Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov. 2009; 8(8):627–644.

[20] Myers AP, Cantley LC. Targeting a common collaborator in cancer development. Sci Transl Med. 2010; 2(48):48ps45.

[21] Liu Z, Li X, Simoneau AR, Jafari M, Zi X. Rhodiola rosea extracts and salidroside decrease the growth of bladder cancer cell lines via inhibition of the mTOR pathway and induction of autophagy. Mol Carcinog. 2012; 51(3):257–267.

[22] Sanguedolce F, Cormio A, Bufo P, Carrieri G, Cormio L. Molecular markers in bladder cancer: Novel research frontiers. Crit Rev Clin Lab Sci. 2015; 52(5):242–55.

[23] Papanas N, Maltezos E, Mikhailidis DP. Metformin and cancer: li¬cence to heal? Expert Opin Investig Drugs. 2010; 19(8):913–917.

[24] Zhang T, Wang X, He D, Jin X, Guo P. Metformin sensitizes human bladder cancer cells to TRAIL-induced apoptosis through mTOR/S6K1-mediated downregulation of c-FLIP. Anticancer Drugs. 2014; 25(8):887–897.

[25] Le Roith D, Baserga R, Helman L, Roberts CT. Insulin-like growth factors and cancer. Ann Intern Med. 1995; 122(1):54–59.

[26] Zhang H, Pelzer A, Kiang DT, Yee D. Down-regulation of type I insulin-like growth factor receptor increases sensitivity of breast cancer cells to insulin. Cancer Res. 2007; 67(1):391–397.

[27] Clemmons DR, Maile L, Ling Y, Yarber J, Busby WH. Role of the integrin alphaVbeta3 in mediating increased smooth muscle cell responsiveness to IGF-I in response to hyperglycemic stress. Growth Horm. IGF Res. 2007; 17(4):265–270.

[28] Kisfalvi K, Eibl G, Sinnett-Smith J, Rozengurt E. Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. Cancer Res. 2009; 69(16):6539–6545.

[29] Dowling RJ, Niraula S, Stambolic V, Goodwin PJ. Metformin in cancer: translational challenges. J. Mol. Endocrinol. 2012; 48(3):31–43.

[30] Pierotti MA, Berrino F, Gariboldi M, Melani C, Mogavero A, et al. Targeting metabolism for cancer treatment and prevention: metformin, an old drug with multi-faceted effects. Oncogene. 2013; 32(12):1475–1487.

[31] Tosca L, Rame´ C, Chabrolle C, Tesseraud S, Dupont J. Metformin decreases IGF1-induced cell proliferation and protein synthesis through AMP-activated protein kinase in cultured bovine granulosa cells. Reproduction. 2010; 139(2):409–418.

[32] Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signaling pathways: insights into insulin action. Nat Rev Mol Cell Biol. 2006; 7(2):85–96.

[33] Vena JE, Freudenheim J, Graham S, Marshall J, Zielezny M, et al. Coffee, cigarette smoking, and bladder cancer in western New York. Ann Epidemiol. 1993; 3(6):586–591.

[34] Dunn SE, Kari FW, French J, Leininger JR, Travlos G, et al. Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. Cancer Res. 1997; 57(21):4667–4672.

[35] Zhao H, Grossman HB, Spitz MR, Lerner SP, Zhang K, et al. Plasma levels of insulin-like growth factor-1 and binding protein-3, and their association with bladder cancer risk. J. Urol. 2003; 169(2):714–717.

[36] Wang Y, Luo H, Li Y, Chen T, Wu S, et al. hsa-miR-96 up-regulates MAP4K1 and IRS1 and may function as a promising diagnostic marker in human bladder urothelial carcinomas. Mol Med Rep. 2012; 5(1):260–265.

[37] Ben Sahra I, Laurent K, Giuliano S, Larbret F, Ponzio G, et al. Targeting cancer cell metabolism: the combination of Metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. Cancer Res. 2010; 70(6):2465–2475.

[38] Yasmeen A, Beauchamp MC, Piura E, Segal E, Pollak M, et al. Induction of apoptosis by metformin in epithelial ovarian cancer: involvement of the Bcl-2 family proteins. Gynecol Oncol. 2011; 121(3):492–498.

[39] Zhuang Y, Miskimins WK. Metformin induces both caspase-dependent and poly (ADP-ribose) polymerase-dependent cell death in breast cancer cells. Mol Cancer Res. 2011; 9(5):603–615.

[40] Han G, Gong H, Wang Y, Guo S, Liu K. AMPK/mTOR-mediated inhibition of survivin partly contributes to metformin-induced apoptosis in human gastric cancer cell. Cancer Biol Ther. 2015; 16(1):77–87.

[41] Yuan SY , Cheng CL , Ho HC , Wang SS , Chiu KY, et al. Nortriptyline induces mitochondria and death receptor-mediated apoptosis in bladder cancer cells and inhibitsbladder tumor growth *in vivo*. Eur J Pharmacol. 2015; 761:309–320.

[42] Akhtar M, Gallagher L, Rohan S. Survivin: role in diagnosis, prognosis, and treatment of bladder cancer. Adv Anat Pathol. 2006; 13(3):122–126.

[43] Gotlieb WH, Saumet J, Beauchamp MC, Gu J, Lau S, et al. *In vitro* metformin anti-neoplastic activity in epithelial ovarian cancer. Gynecol Oncol. 2008; 110(2):246–250.

[44] Rattan R, Graham RP, Maguire JL, Giri S, Shridhar V. Metformin suppresses ovarian cancer growth and metastasis with enhancement of cisplatin cytotoxicity *in vivo*. Neoplasia. 2011; 13(5):483–491.

[45] Erices R, Bravo M, Gonzalez P, Oliva B, Racordon D, et al. Metformin, at concentrations corresponding to the treatment of diabetes, potentiates the cytotoxic effects of carboplatin in cultures of ovarian cancer cells. Reprod Sci. 2013; 20(12):1433–1446.

[46] Hanna RK, Zhou C, Malloy KM, Sun L, Zhong Y, et al. Metformin potentiates the effects of paclitaxel in endometrial cancer cells through inhibition of cell proliferation and modulation of mTOR pathyway. Gynecol. Oncol. 2012; 125(2):458–469.

[47] Chan DK, Miskimins WK. Metformin and phenethyl isothiocyanate combined treatment *in vitro* is cytotoxic to ovarian cancer cultures. J Ovarian Res. 2012; 5(1):19.

[48] Rattan R, Graham RP, Maguire JL, Giri S, Shridhar V. Metformin suppresses ovarian cancer growth and metastasis with enhancement of cisplatin cytotoxicity *in vivo*. Neoplasia. 2011; 13(5):483–491.

[49] Rocha GZ, Dias MM, Ropelle ER, Osório-Costa F, Rossato FA, et al. Metformin amplifies chemotherapy-induced AMPK activation and antitumoral growth. Clin Cancer Res. 2011; 17():3993–4005.

[50] Iliopoulos D, Hirsch HA, Struhl K. Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. Cancer Res. 2011; 71(9):3196–3201.

[51] Morgillo F, Sasso FC, Della Corte M, Vitagliano D, D'Aiuto E, et al. Synergistic effects of metformin treatment in combination with gefitinib, a selective EGFR tyrosine kinase inhibitor, in LKB1 wild-type NSCLC cell lines. Clin Cancer Res. 2013; 19(13):3508–3519.

[52] Zhang Y, Guan M, Zheng Z, Zhang Q, Gao F, et al. Effects of metformin on CD133+ colorectal cancer cells in diabetic patients. PLoS One. 2013; 8(11):e81264.

[53] Garcia-Canton C, Anadón A, Meredith C. γH2AX as a novel endpoint to detect DNA damage: applications for the assessment of the *in vitro* genotoxicity of cigarette smoke. Toxicol *In vitro*. 2012; 26(7):1075–1086.

[54] Halicka HD, Zhao H, Li J, Traganos F, Zhang S, et al. Genome protective effect of metformin as revealed by reduced level of constitutive DNA damage signaling. Aging. 2011; 3(10):1028–1038.

[55] Algire C, Moiseeva O, Deschenes-Simard X, Amrein L, Petruccelli L, et al. Metformin reduces endogenous reactive oxygen species and associated DNA damage. Cancer Prev Res (Phila). 2012; 5(4):536–543.

[56] Bost F, Ben-Sahra I, Tanti JF. Prevention of mutagenesis: new potential mechanisms of metformin action in neoplastic cells. Cancer Prev Res (Phila). 2012; 5(4):503–506.

[57] Bowman KJ, Al-Moneef MM, Sherwood BT, Colquhoun AJ, Goddard JC, et al. Comet assay measures of DNA damage are predictive of bladder cancer cell treatment sensitivity *in vitro* and outcome *in vivo*. Int J Cancer. 2014; 134(5):1102–1111.

[58] Camargo EA, da Silva GN, Gobette CP, Marcondes JP, Salvadori DM. No relationship between the amount of DNA damage and the level of hMLH1 and RASSF1A gene expression in bladder cancer cells treated with cisplatin and gemcitabine. Asian Pac J Cancer Prev. 2013; 14(10):5941–5948.

[59] Garcia EY. Fluamine, a new synthetic analgesic and antiflu drug. J Philipp Med Assoc. 1950; 26(7):287–293.

[60] Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature. 2009; 460(7251):103–107.

[61] Ropelle ER, Pauli JR, Zecchin KG, Ueno M, de Souza CT, et al. A central role for neuronal adenosine 5'-monophosphate-activated protein kinase in cancer-induced anorexia. Endocrinology. 2007; 148(11):5220–5229.

[62] Horn T, Laus J, Seitz AK, Maurer T, Schmid SC, et al. The prognostic effect of tumour-infiltrating lymphocytic subpopulations in bladder cancer. World J Urol. 2016; 34(2):181–187.

[63] Danielle G, Sandra B, Kay F. Autophagy: cellular and molecular mechanisms. J Pathol. 2010; 221(1):3–12.

[64] Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol. 2011; 13(2):132–141.

[65] Masini M, Bugliani M, Lupi R, del Guerra S, Boggi U, et al. Autophagy in human type 2 diabetes pancreatic beta cells. Diabetologia. 2009; 52(6):1083–1086.

[66] He C, Zhu H, Li H, Zou MH, Xie Z. Dissociation of Bcl-2-Beclin1 complex by activated AMPK enhances cardiac autophagy and protects against cardiomyocyte apoptosis in diabetes. Diabetes. 2013; 62(4):1270–1281.

[67] Xie Z, Lau K, Eby B, Lozano P, He C, et al. Improvement of cardiac functions by chronic metformin treatment is associated with enhanced cardiac autophagy in diabetic OVE26 mice. Diabetes. 2011; 60(6):1770–1778.

[68] Song YM, Lee YH, Kim JW, Ham DS, Kang ES, et al. Metformin alleviates hepatosteatosis by restoring SIRT1-mediated autophagy induction via an AMP-activated protein kinase-independent pathway. Autophagy. 2015; 11(1):46–59.

[69] Ng F, Tang BL. Sirtuins' modulation of autophagy. J Cell Physiol. 2013; 228(12):2262–2270.

[70] Takahashi A , Kimura F , Yamanaka A , Takebayashi A , Kita N, et al. Metformin impairs growth of endometrial cancer cells via cell cycle arrest and concomitant autophagy and apoptosis. Cancer Cell Int. 2014; 16:14–53.

[71] Yan S, Yang X, Chen T, Xi Z, Jiang X. The PPARγ agonist Troglitazone induces autophagy, apoptosis and necroptosis in bladder cancer cells. Cancer Gene Ther. 2014; 21(5):188–193.

[72] Liu Z, Antalek M, Nguyen L, Li X, Tian X, et al. The effect of gartanin, a naturally occurring xanthone in mangosteen juice, on the mTOR pathway, autophagy, apoptosis, and the growth of human urinary bladder cancer cell lines. Nutr Cancer. 2013; 65:68–77.