Indonesian Journal of Clinical Pharmacy, June 2016
Vol. 5 Iss. 2, pg 75–83
ISSN: 2252–6218
Available online at:
http://ijcp.or.id
DOI: 10.15416/ijcp.2016.5.2.75
Research Article

Metformin Enhances Anti-proliferative Effect of Cisplatin in Cervical Cancer Cell Line

Ratih D. Yudhani[1], Riza N. Pesik[2], Dono Indarto[3]
[1]Department of Pharmacology, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia
[2]Department of Pathology, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia
[3]Department of Physiology, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia

Abstract
Cervival cancer is one of the top rank of gynecological malignancy in the world, leading to high morbidity and mortality rates. Cisplatin is a chemotherapeutic agent that is generally used to treat cervical cancer but the use of this drug is limited because of serious side effects. Metformin, a diabetic drug, decreases not only blood glucose levels but also cell viability of some cancer cells. The aim of this study was to investigate the anti-proliferative effect of combination metformin and cisplatin in HeLa cells (cervical cancer cell line). Anti-proliferative effect of these combined drugs was analized using MTT assay, combination index assay and HeLa cell morphology. Inhibitory concentration (IC_{50}) of cisplatin and metformin was determined before performing combination index assay. Administration of 10 mM metformin showed inhibition of HeLa cell proliferation and it reached 50% inhibition of cell proliferation at 60 mM. Whilst, cisplatin showed a stronger anti-proliferative effect with initial inhibition dose at 12 µM and IC_{50} dose at 44 µM. Combination of 30 mM metformin and 5 µM cisplatin indicated the strongest anti-proliferative effect on HeLa cell. In conclusion, metformin may become a promising drug for treatment of cervical cancer in future which enhances anti-proliferative effect of cisplatin.

Key words: Anti-proliferative effect, combination index, cervical cancer, cisplatin, metformin

Peningkatan Efek Anti-poliferatif Cisplatin oleh Metformin pada *Cell Line* Kanker Serviks

Abstrak
Kanker serviks merupakan salah satu keganasan ginekologi tertinggi di dunia, dengan tingkat morbiditas dan mortalitas yang tinggi. Cisplatin merupakan obat kemoterapi yang umum digunakan untuk terapi kanker serviks, namun penggunaannya relatif terbatas karena menyebabkan beberapa efek samping yang serius. Metformin merupakan obat anti diabetik yang mampu menurunkan kadar glukosa darah dan juga mampu menurunkan viabilitas beberapa jenis sel kanker. Penelitian ini bertujuan untuk mengetahui efek anti-proliferasi kombinasi metformin dan cisplatin pada sel HeLa (*cell line* kanker serviks). Efek anti-proliferasi kombinasi kedua senyawa tersebut dianalisis melalui MTT *assay*, *combination index assay* dan morfologi sel HeLa. Nilai *inhibitory concentration* (IC_{50}) metformin dan cisplatin pada sel HeLa ditentukan lebih dahulu sebelum melakukan *combination index assay*. Pemberian metformin 10 mM mulai menunjukkan penghambatan proliferasi sel HeLa dan penghambatan proliferasi sel mencapai 50% pada dosis 60 mM. Cisplatin menunjukkan efek anti-proliferasi yang lebih kuat dengan dosis awal penghambatan sebesar 12 µM dan IC_{50} sebesar 44 µM. Kombinasi antara metformin 30 mM dan cisplatin 5 µM memperlihatkan efek anti-proliferatif terkuat pada sel HeLa. Sebagai kesimpulan, metformin kemungkinan menjadi obat yang menjanjikan untuk terapi kanker serviks di masa mendatang dengan cara meningkatkan efek anti-proliferasi cisplatin.

Kata kunci: Cisplatin, *combination index*, efek anti-proliferatif, kanker serviks, metformin

Correspondence: Ratih D. Yudhani, dr., M.Sc, Department of Pharmacology, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia, email: rdyudhani@gmail.com
Received: 11th June 2015, Accepted: 3rd September 2015, Published: 1st June 2016

Introduction

Cervical cancer is the second highest gynecological malignancy around the world, which the incidence rate is approximately a half million per year and the mortality rate is nearly 275 million per year.[1] In Indonesia, however, cervical cancer remains the first highest gynecological malignancy and still becomes a reproductive health problem. New cases of this cancer are estimated around 40 million per year.[2]

Chemotherapy is one of the main therapies for inhibition of cervical cancer cells. Cisplatin, a platinum-containing drug, has recently been recommended as the first line chemotherapeutic agent for treating advanced cervical cancer.[3] However, administration of this drug frequently results in serious adverse effects such as neurotoxicity, nephrotoxicity and bone marrow suppression.[3,4] Although cisplatin exhibits a growth inhibitory activity, this drug shows an individual response and high resistance in some patients with cervical cancer.[5]

Metformin or N', N'-dimethylbiguanide is recognized as a standard drug for diabetes type 2, which reduces blood glucose levels with minimally hypoglycemic effects and minimal risk for lactic acidosis.[6] In addition to the diabetic activity, metformin also exhibits anticancer activity via activation of the AMP-activated protein kinase pathway, modulation of mTOR signaling, and inhibition of cell division.[7–12] Recent studies have indicated that administration of metformin can inhibit proliferation of some cancer cells in the breast, prostate, colon, endometrium, ovary and brain in different mechanisms of action.[13–16] Moreover, metformin administered to cervical cancer cell line (HeLa) induces apoptosis in dose dependent manner with the IC_{50}, 60 mM.[17]

In the last decade, development of cancer therapy has provided an opportunity to combine a chemotherapy agent with other drugs in order to enhance its therapeutic effect and to minimize its adverse effects. Metformin for instance may enhance anti-proliferative effect of some chemotherapy drugs. Recent studies have reported that combination of doxorubicin (cytotoxic agent) and metformin is able to kill various breast and thyroid cancer cells including cancer stem cells.[18,19] Therefore, the aim of this study was to investigate anti-proliferative effect of combination of metformin and cisplatin in HeLa cancer cells.

Methods

All chemicals used in this study were obtained from Invitrogen unless otherwise stated. Cisplatin solution was purchased from Kalbe Farma, Jakarta. HeLa cell line was obtained from cell stocks at Parasitology Laboratory, Faculty of Medicine, University of Gadjah Mada, Yogyakarta. Materials required for growing the HeLa cell such as DMEM powder, fetal bovine serum, fungizone, penstrep and 0,25% (w/v) trypsin EDTA were purchased from Gibco whilst 4-(2-hydrocyethyl)-piperazine-ethane) sulphonic acid (HEPES) was from Sigma Aldrich, USA and bicarbonate sodium was from Nacalai Tesque. Phosphate Buffer Saline solution was purchased from Invitrogen. Sodium dodecyl sulphate, chloric acid and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) used for proliferation assay was obtained from Merck, Germany.

Cell Culture

HeLa cell line was grown in DMEM supplemented with 10% (v/v) heat-inactivated FBS, 2% (v/v) penstrep and 0.5% (v/v) fungizone. Cells were maintained at 37 ^{0}C in a humidified incubator (Heraeus HERA cell)

with 5% CO2 atmosphere.

Inhibitory Concentration (IC_{50}) of Cisplatin and Metformin

Cisplatin solution was diluted in a complete cell medium to get 100, 50, 25, 12, 6, 3, 1.5 and 0.75 µM final concentration. Meanwhile, a series of metformin dilution (80, 40, 20, 10, 5 and 2.5 mM) was performed by adding the complete cell medium as well. The complete cell medium served as zero concentration of cisplatin or metformin dilution. Once HeLa cell culture has reached 80% confluence, it was seeded in a 96 well plate (SPL®) and incubated at 37 ^{0}C for 24 hours. Following day, cell medium was replaced with fresh DMEM added with the various concentrations of diluted cisplatin or metformin.

MTT proliferation assay

Harvested HeLa cells were seeded into a 96 well plate with 1x10^4 cell concentration/well. The HeLa cell was put into three wells and the fourth well contained medium only which served as a negative control. Seeded cells were then incubated at 37 ^{0}C in a 5% CO_2 incubator and cells were treated with 0.05% (w/v) MTT after 6 hour time point. After that, cells were added with 20% (w/v) SDS in 20 mM HCl and incubated at room temperature for overnight before reading it in a Bio-Rad spectrophotometer with optical density 595 nm.

Combination index assay

Cisplatin solution was diluted in the complete cell medium to get 5, 10, 15 and 30 µM final concentration while metformin was diluted in the same medium to get 7,5; 15; 22,5 dan 30 mM concentration. Once HeLa cell culture has reached 80% confluence, it was seeded into a 96 well plate and incubated at 37 ^{0}C for 24 hours. Following day, cell medium was replaced with fresh DMEM added with various doses of combination of diluted metformin and cisplatin. According to Cancer Chemoprevention Research Center (2009), combination of two chemicals has a synergetic effect if its combination index (CI) value is 0.3–0.7 and 1.45–2.33 CI value for antagonist effect.[20]

Data analysis

Percentage of cell proliferation was calculated by using formula from Cancer Chemoprevention Research Center.[21]

$$\frac{(Treatment\ absorbance - Medium\ only\ absorbance)}{(Control\ cell\ absorbance - Medium\ only\ absorbance)} x\ 100\%$$

IC_{50} cisplatin was determined by using regression linear equation (y = bx + a) between logarithm of cisplatin concentration and percentage of cell proliferation. Combination index (CI) of cisplatin and metformin was calculated by using formula[22]:

$$CI = \frac{D1}{Dx1} + \frac{D2}{Dx2}$$

where D1 and D2 were combined dosages of cisplatin and metformin to obtain 50% inhibition of cell growth whereas Dx1 and Dx2 were individual dose of cisplatin or metformin for obtaining 50% inhibition of cell growth.

Results

In this study, cell proliferation assay was used to evaluate the anti-proliferative effect of metformin and cisplatin alone in HeLa cell line. A similar pattern of inhibition of cell growth was observed in HeLa cells treated with both drugs (Figure 1A&B). Initial inhibition of cell growth appeared in HeLa cells treated with 10 mM metformin and 12 µM cisplatin. By increasing drug doses, higher inhibition of cell proliferation was observed in HeLa cell treated with cisplatin compared to HeLa cell treated with metformin. Meanwhile, administration of 60 mM metformin and 44 µM cisplatin could

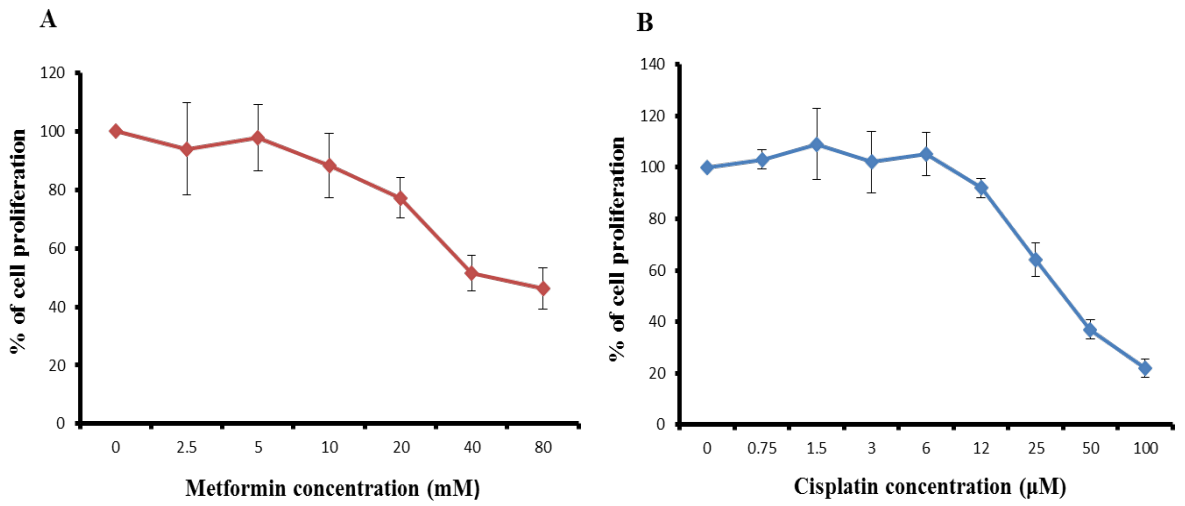

Figure 1 Proliferation Rate of HeLa Cells Treated with Various Doses of Metformin and Cisplatin
HeLa cells with 1 x 10^4 cell concentration/well were cultured into a 96 well plate for 24 hours. Following day, the HeLa cells were treated with (A) 0-80 mM metformin and (B) 0–100 µM cisplatin. Cell proliferation was determined by using MTT proliferation assay. Data were presented in means±SD in three independent experiments

inhibit 50% HeLa cell growth respectively.

CI assay was then performed to evaluate whether or not co-administration of metformin in HeLa cell treated with cisplatin has a synergistic effect. In Table 1, administration of various doses of metformin and cisplatin in HeLa cells showed different effects. Lower doses of metformin (7.5 and 15 mM) appeared to have variable effects to HeLa cell when combining with 5–30 µM cisplatin. In the lowest dose of cisplatin, addition of 7.5 or 15 mM metformin in HeLa cells showed a border line effect. Lower doses of metformin combined with 10, 15 or 30 µM cisplatin had an antagonist effect in HeLa cells except combination of 7.5 mM metformin and 30 µM cisplatin had a synergetic effect. Whilst, a synergetic effect was observed in HeLa cell treated with higher doses (22.5 and 30 mM) of metformin and 5–30 µM cisplatin.

Based on differential effects of administration of metformin and cisplatin in HeLa cell, we microscopically evaluated the HeLa cell proliferation. Figure 2 demonstrated that inhibition of cell proliferation was observed in HeLa cells treated with combination of metformin and cisplatin. A higher anti-proliferative effect was found in HeLa cell treated with 7.5 mM metformin and 10 µM cisplatin, compared with untreated HeLa cells (Fig.2A&B). Moreover, dead HeLa cells were detected among viable HeLa

Table 1 CI Value of Administration of Metformin and Cisplatin in HeLa cells

Metformin (mM)	Cisplatin (µM)			
	5	10	15	30
7.5	0.93	2.32**	1.19	0.73
15	0.97	1.53	1.52	1.41
22.5	0.36	0.56	0.51	0.64
30	0.34*	0.36	0.69	0.48

*lower CI value ; ** higher CI value

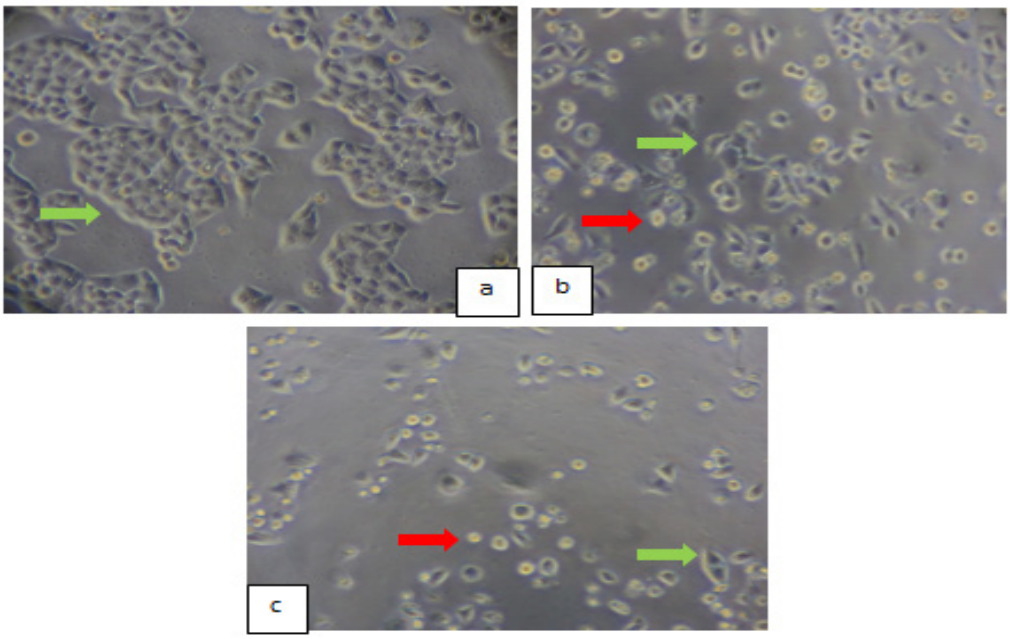

Figure 2 Morphology of HeLa Cells was Treated with Co-administration of Metformin and Cisplatin for 24 hour Incubation

(a) HeLa cell with no treatment of metformin and cisplatin (negative control). (b) HeLa cell was incubated with a combination of 7.5 mM metformin and 10 µM cisplatin and (c) 30 mM metformin and 5 µM cisplatin respectively. Images were generated by using a light microscope with 100x magnification. Green arrow indicated viable cells and red arrow indicated dead cells

cells. Meanwhile, administration of 30 mM metformin and 5 µM cisplatin in HeLa cell has higher inhibition of cell proliferation than administration of 7.5 mM metformin and 10 µM cisplatin and negative control (Figure 2A-C). In addition, dead cells are as equal as viable cells (Figure 2C).

Discussion

Chemotherapeutic agents which are administered alone or combination have been considered as the first line therapy for inhibiting growth of some cancer cells. We have demonstrated that metformin is able to inhibit HeLa cell proliferation in dose dependent manner. Because IC_{50} metformin is high, it is unlikely to a administer metformin alone to avoid its side effects and toxicity. In addition during this study administration of 30 mM metformin and 5 µM cisplatin has a better inhibitory effect against HeLa cells, compared with administration of 7.5 mM metformin and 10 µM cisplatin. We speculate that the different effect of metformin administration may suggests that this drug has a different mechanism of action.

Anticancer activity of metformin varies in some cancer cells. As reported by Xiao et al. (2012), three cervical cancer cell lines (C33A, CaSki, and Me180) have IC_{50} less than 10 mM and other cervical cancer cells (HeLa, MS751, and HT-3) have IC_{50} more than 20 mM after 72 hour incubation.[8] Interestingly, another study has indicated that administration of 0.1 mM metformin for 24 hours is able to supress 50% of HeLa cell growth.[23] An inhibitory effect of metformin is also observed in some thyroid cancer cell lines, which incubated with various doses of metformin for 24–72 hours.[18] During 24 hour incubation, a higher dose of metformin (≥40 mM) was required for inhibition of cell proliferation of thyroid cancer cell lines.

After 48–72 hour incubation, administration of 20 mM metformin reduced cell viability of thyroid cancer cell lines up to 50% or more. In liver cancer cell line (Hep-G2), administration of 20 mM metformin also inhibits 50% of cancer cell growth.[24] The IC_{50} metformin used in these studies is lower than that of in our study. The discrepancy of these results might be related to cancer cell type, high number passage, incubation time and complexity of metformin biological effects.

In the present work, we demonstrated that metformin induced cell death and enhanced the anti-proliferative effect of cisplatin in HeLa cell line (Figure 2). It might be through the same pathway as demonstrated in thyroid cancer cell line and in ovarian cancer cell line or the different pathway as observed in breast cancer cell line.[18,25,26] Induction of cell death by metformin is partly mediated by activation of caspace 3 in the intrinsic pathway.[18,25] Whereas in breast cancer cells treated with metformin, cell death occurred via ERK signaling, which increases expression of p53 and Bax family.[26]

Interestingly, all of our data suggest that combination of the highest dose of metformin and the lowest dose of cisplatin is more effective to inhibit cell growth and to induce cell death than combination of other doses of metformin and cisplatin. In line with this finding, a recent study has demonstrated that administration of 10 mM metformin and 5 μM cisplatin decreases effectively cell viability of thyroid cancer, compared with administration of 5 μM cisplatin alone.[18] Overall, this indicates that metformin exerts its anti-proliferative effect in HeLa cells by inducing cell death.

Metformin administered in gynecological cancer cells also modulates energy metabolism through activation of AMP-activated protein kinase (AMPK) and inhibition of mammalian target of rapamycin (mTOR), leading to reduction of protein synthesis, apoptosis and autophagy.[27,28] Xiao and her colleagues reported that administration of metformin induces apoptosis and autophagy in some cervical cancer cell lines excluding HeLa cell via AMPK activation in the presence of tumour suppressor LKB1.[8] A recent study has also demonstrated that metformin-activated AMPK reduces not only cell proliferation and protein synthesis but also angiogenesis and metastases in nude mice injected with ovarian cancer cells.[28] Therefore, further investigation is required whether or not the AMPK/mTOR pathway implicates in cell death in HeLa cells of our study, which were treated with combination of metformin and cisplatin.

Although combined treatment with metformin (>22.5 mM) and cisplatin (5–30 μM) had synergistically anti-proliferative effects on HeLa cells, the antagonist effect of both drugs was observed in HeLa cells with metformin dosages are lower than or equal 15 mM (Table 1 and Figure 2). The similar effect of this combined treatment is also reported in gastric and brain cancer cells.[30,31] Treatment with 5 or 10 mM metformin and 5 μM cisplatin diminished inhibition of cell growth and induction of apoptosis in the human gastric cancer cell line (MKN-45).[30] Besides this findings, researchers also documented that the antagonistic effect of these drugs is related to overexpression of survivin, mTOR and protein kinase B (Akt).[30] Up regulation of these genes and proteins are also found in cancer cell line in the brain and other gastric cancer cell lines.[31,32]

Therefore, it is not surprising if survivin and Akt are essential proteins for mediating cisplatin resistance in gastric cancer.[32] In order to elucidate antagonistic property of combinational therapy of metformin and cisplatin in HeLa cells, investigation of survivin, mTOR and Akt is required. Moreover, metformin-induced cell death in HeLa cells might partly be through AMPK independent pathway.

Conclusions

Metformin may become an adjuvant therapy, which works synergistically with cisplatin to treat cervical cancer even though combination of ≤15 mM metformin and cisplatin results in antagonist effects. Analysis of cell cycle and apoptosis and Western blotting will be useful for addressing mechanism of metformin action in cervical cancer cells in future. Expression of survivin, mTOR and Akt genes or proteins are very important for determination of antagonism of this combined therapy.

Acknowledgments

The authors would like to thank Sebelas Maret University for supported the research grant and also Rumbiwati for her technical assistance in conducting this research.

Funding

This work was supported by a research grant from PNBP, Sebelas Maret University, Surakarta with grant number 858/UNS27.11/PN/2014.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90. doi: 10.3322/caac.20107
2. Rasjidi I. Epidemiologi kanker serviks. Indones J Cancer. 2009;3:103–8.
3. Koh WJ, Greer BE, Abu-Rustum NR, Apte SM, Campos SM, Chan J, et al. Cervical cancer. J Natl Compr Canc Netw. 2013;11:320–43.
4. Florea AM, Büsselberg D. Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. Cancers. 2011;3:1351–71. doi:10.3390/cancers3011351
5. 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;71:3196–7. doi: 10.1158/0008-5472.CAN-10-3471
6. Sahra IB, Brustel YLM, Tanti JF, Frederic B. Metformin in cancer therapy: a new perspective for an old antidiabetic drug?. Mol Cancer Ther. 2010;9:1092–9. doi: 10.1158/1535-7163.MCT-09-1186
7. Rattan R, Giri S, Hartmann LC, Shridhar V. Metformin attenuates ovarian cancer cell growth in an AMP-kinase dispensable manner. J Cell Mol Med. 2011;15:166–78. doi: 10.1111/j.1582-4934.2009.00954.x
8. Xiao X, He Q, Lu C, Werle KD, Zhao RX, Chen J, et al. Metformin impairs the growth of liver kinase B1-intact cervical cancer cells. Gynecol Oncol. 2012;127:249–55. doi:10.1016/j.ygyno.2012.06.032
9. Kwan HT, Chan DW, Cai PCH, Mak CSL, Yung MMH, Leung THY, et al. AMPK activators suppress cervical cancer cell growth through inhibition of DVL3 mediated Wnt/b-Catenin signaling activity. Plos One. 2013;8:53597. doi: 10.1371/journal.pone.0053597
10. Yung MMH, Chan DW, Liu VWS, Yao KM, Ngan HYS. Activation of AMPK inhibits cervical cancer cell growth through AKT/FOXO3a/FOXM1 signaling cascade. BMC Cancer. 2013;13:327. doi: 10.1186/1471-2407-13-327
11. Hanna RK, Zhou C, Malloy KM, Sun L,

Zhong Y, Gehrig PA, et al. Metformin potentiates the effects of paclitaxel in endometrial cancer cells through inhibition of cell proliferation and modulation the mTOR pathway. Gynecol Oncol. 2012;125:458–69. doi: 10.1016/j.ygyno.2012.01.009

12. Hu T, Chung YM, Guan M, Ma M, Ma J, Berek JS, et al. Reprogramming ovarian and breast cancer cells into non-cancerous cells by low-dose metformin or SN-38 through FOXO3 activation. Scientific Reports. 2014;4:5810. doi: 10.1038/srep05810
13. Evans JM, Emslie-Smith AM. Metformin and reduced risk of cancer in diabetics patients. BMJ. 2005;330:1304–5. doi: 10.1136/bmj.38415.708634.F7
14. Dowling RJ, Goodwin PJ, Stambolic V. Understdaning the benefit of metformin use in cancer treatment. BMJ Med. 2011;9:2–6. doi: 10.1186/1741-7015-9-33
15. Quinn BJ, Kitagawa H, Memmot RM, Gills JJ, Dennis PA. Repositioning metformin for cancer prevention dan treatment. Trends Endocrinol Metab. 2013;30:1–12. doi: 10.1016/j.tem.2013.05.004
16. Vallianou NG, Evangelopoulos A, Kazazis C. Metformin and cancer. Rev Diabet Stud. 2013;10:228–35. doi: 10.1900/RDS.2013.10.228
17. Yudhani RD. Pengaruh metformin terhadap penekanan ekspresi cyclin D1, aktivasi p53 dan induksi apoptosis pada kultur sel HeLa (tesis). Yogyakarta: Program Studi Ilmu Kedokteran Dasar dan Biomedis, Fakultas Kedokteran, Universitas Gadjah Mada; 2014.
18. Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. Cancer Res. 2009;69:7506–11. doi: 10.1158/0008-5472.CAN-09-2994
19. Chen G, Xu S, Renko K, Derwahl M. Metformin inhibits growth of thyroid carcinoma cells, suppresses self-renewal of derived cancer stem cells, and potentiates the effect of chemotherapeutic agents. J Clin Endocrinol Metab. 2012;97:510–20.doi:10.1210/jc.2011-1754
20. Cancer-Chemoprevention-Research-Center (CCRC). Prosedur tetap uji kombinasi dengan agen kemoterapi. Yogyakarta: Fakultas Farmasi UGM; 2009.
21. Cancer Chemoprevention Research Center (CCRC). Prosedur tetap uji sitotoksisitas metode MTT. Yogyakarta: Fakultas Farmasi UGM; 2009.
22. Reynolds CP, Maurer BJ. Evaluating response to antineoplastic drug combinations in tissue culture models. Methods Mol Med. 2005;110:173–83. doi: 10.1385/1-59259-869-2:173
23. Gao S, Jiang J, Li P, Song H, Wang W, Li C, et al. Attenuating tumour angiogenesis: a preventive role of metformin against breast cancer. Biomed Research Int. 2014:1–6. doi: 10.1155/2015/592523
24. Xiong Y, Lu JQ, Zhao J, Wu GY. Metformin inhibits growth of hepatocellular carcinoma cells by inducing apoptosis via mitochondrion-mediated pathway. Asian Pac J Cancer Prev. 2012;13:3275–9.
25. Yasmeen A, Beauchamp MC, Piura E, Segal E, Pollak M, Gotlieb WH. Induction of apoptosis by metformin in epithelial ovarian cancer: involvement of the Bcl-2 family proteins. Gynecol Oncol. 2011;121:492–8. doi: 10.1016/j.ygyno.2011.02.021
26. Malki A, Youssef A. Antidiabetic drug metformin induces apoptosis in human MCF breast cancer via targeting ERK signaling. Oncol Res Featur Preclin Clin Cancer Ther. 2011;19:275–85. doi: 10.3727/096504011X13021877989838

27. Dowling RJO, Niraula S, Stambolic V, Goodwin PJ. Metformin in cancer: translational challenges. J Mol Endocrinol. 2012;48:R31–R43. doi: 10.1530/JME-12-0007
28. Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, et al. AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. Cell Metab. 2013;17:113–24. doi: 10.1016/j.cmet.2012.12.001
29. Rattan R, Graham RP, Maguire JL, Giri S, Shridhar V. Metformin supresses ovarian cancer cell growth and metastases with enhancement of cysplatin cytotoxicity in vivo. Neoplasia. 2011;13:483–91.
30. Lesan V, Ghaffari SH, Salaramoli J, Heidari M, Rostami M, Alimoghaddam K, et al. Evaluation of antagonistic effects of metformin with cisplatin in gastric cancer cells. Int J Hematol Oncol Stem Cell Res. 2014;8:12–9.
31. Janjetovic K, Vucicevic L, Misirkic M, Vilimanovich U, Tovilovic G, Zogovic N, et al. Metformin reduces cisplatin-mediated apoptotic death of cancer cells through AMPK-independent activation of Akt. Eur J Pharmacol. 2011;651:41–50. doi: 10.1016/j.ejphar.2010.11.005
32. Sun XP, Dong X, Lin L, Jiang X, Wei Z, Zhai B, et al. Up-regulation of surviving by AKT and hypoxia-inducible factor 1α contributes to cisplatin resistance in gastric cancer. FEBS Journal. 2014;281:115–28. doi: 10.1111/febs.12577