Metformin May Block the Way Breast Cancer Metastasis: A Meta-analysis and Mechanism Review

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Abstract

Background

Metformin, which is cheap and easy to get, is a first-line anti-hyperglycemia drug. Recently, its anti-tumor effect has been revealed. Here we performed a meta-analysis to summarize previous studies and a narrative review to gather the mechanisms involved in the potential relationship.

Methods

We searched related articles in database of Pubmed, EMbase, Web of science, the Cochrane Library, China National Knowledge Infrastructure (CNKI), the Wanfang and Sinomed and obtained 8 clinic trials that investigated the connection between metformin and breast cancer metastasis, containing 2 randomized controlled trials (RCTs) and 6 retrospective cohort studies. We evaluated each retrospective cohort study by Newcastle-Ottawa Scale (NOS), while RCT by Chcorane Risk of Bias tool. Pooled hazard ratios (HRs), risk ratios (RRs) and we calculated associated 95% confidence intervals (CIs) with a random-effect, generic inverse variance method. We also collected the possible mechanisms of cancer metastasis inhibition from metformin.

Results

A total of 8 studies containing 13919 breast cancer patients without distant metastasis before they got anticancer treatment. The result showed that adjuvant metformin in treatment of local breast cancer facilitated to suppress metastasis (HR = 0.69, 95% CI = 0.57–0.82, $p < 0.0001$, $I^2 = 0\%$), and the result was consistent with the subgroup of breast cancer patients with type 2 diabetes mellitus (T2DM) (HR = 0.68, 95% CI = 0.57–0.82, $p < 0.0001$, $I^2 = 0\%$).

Conclusion

The meta-analysis suggested metformin might repress the metastasis and be benefit to distant metastasis-free survival (DMFS) when added to systemic breast cancer therapy, supporting anti-tumor effects of metformin on breast cancer.

Background

Breast cancer has a high incidence in female tumors, which affects the health of women around the world. In a sample survey conducted in mainland China in 2019, women with T2DM had an increased risk of breast cancer [1]. A lot of clinical and epidemiological evidences have linked hyperinsulinemia, insulin resistance, and diabetes to poor breast cancer outcomes [2]. In addition, early breast cancer patients with T2DM had an increased likelihood of recurrence and metastasis [3, 4], implicating that T2DM might be a dependent risk factor of breast cancer prognosis. There are various treatments for early breast cancer, but for breast cancer with distant metastasis, the effective method is still a difficulty. Therefore, prevention breast cancer patients from metastasis is economical and available.

Metformin, extracted from *Galega* officinalis (the French lilac) [5], is an anti-hyperglycemia drug and insulin sensitizer used in the therapy of T2DM, a chronic disease characterized by insulin resistance [6]. In recent years, metformin has also been reported to reduce cancer risk and improve clinical outcomes [7, 8]. This drug inhibits tumor growth and cell proliferation in most breast cancer subtypes in vitro at different degrees [9–12]. Furthermore, the metformin targets to breast cancer stem cells (BCSCs), reduces tumor mass and prolongs remission with doxorubicin [13]. Based on these basic studies, various of clinic studies were conducted to explore whether adjuvant metformin may contribute to the prognosis of breast cancer patients [14, 15].

We here provided a meta-analysis to explore whether metformin adjuvant therapy could reduce breast cancer metastasis and prolonged the distant metastasis-free survival (DMFS). We also collected the probable mechanisms about metformin. We hoped this study might help to deep comprehension of the role of metformin in cancer treatment.

Methods

Study selection

We searched all published articles up to May 2020 in database of Pubmed, EMbase, Web of science, Cochrane Library and Chinese database of CNKI, WanFang and Sinomed for relevant studies. The keywords for searching included “metformin”, “breast neoplasms”, “therapies, drug” and “neoplasm metastasis”. Contralateral axillary lymph node metastasis is controversial in the clinical stage of breast cancer [16]. We here included “lymphatic metastasis” in our search terms as well. After that, the selected studies were manually screened and only human subjects and original articles were considered eligible. Selected publications were all in English or Chinese.

Inclusion and exclusion criteria

Since aiming to investigate whether metformin could suppress breast cancer metastasis, we set inclusion and exclusion criteria for the meta-analysis:

i. All patients were diagnosed as primary breast cancer without metastatic disease before they got anticancer treatment;
ii. The breast cancer patients received not only antihyperglycemic therapy;

iii. The patients we included were divided into two group. The “metformin group” was defined as the breast cancer patients who received metformin including combination with other antihyperglycemic drugs during breast cancer therapy, while the “non-metformin group” was defined as patients who did not use metformin but chose other hypoglycemic drugs or they did not received any antihyperglycemic therapy during anti-tumor treatment;
iv. There were various methods to treat breast cancer systematically, thus, we set no limit on the consistency of other treatments except antihyperglycemic therapy;
v. The number of patients who ended with distant metastasis and the number of patients who participated in the studies, or relevant Kaplan-Meier curves, or HRs, RRs with associated 95% CIs between study groups and control groups were provided;
vi. RCT, cross-sectional studies or cohort studies published as original manuscript.

The TNM stage of breast cancer was based on the criteria of American joint committee on cancer (AJCC) 8th edition cancer staging manual and M-phase was considered as “distant metastasis” [17].

Data extraction and quality assessment

Two authors reviewed each selected article independently and extracted relevant data with a structured table. We extracted data containing: authors, year of publication, country which the study was conducted in, study type, sample size, follow-up time, the average or median age of study population, the number of breast cancer patients with T2DM, other treatment than antihyperglycemic therapy, metformin dose, the distant metastasis number and total number of study group and control group/HR/RR. If there was any disagreement, the final decision would be made by the group discussion. Our work was performed according to the Preferred Reporting Items for Systematic Review ang Meta-Analysis Protocols (PRISMA-P) 2015 statement. The quality of retrospective cohort studies we intended to include was assessed with reference to the NOS, which was composed of three parts: selection, comparability and exposure, and only the study score was not less than 6 stars (up to 9 stars) could be accepted [18, 19]. The rest of studies were RCTs, thus we chose Chcorane Risk of Bias tool to assess their quality.

Statistical analyses

If relative RRs and HRs with associated 95% CIs were not available, we would extract the metastasis numbers and total numbers in study group and control group, then used STATA14.0 (Stata, College Station) to calculated RRs and associated 95% CI. As the articles published before, the distinction between relative RR and HR was ignored [20, 21]. HR/RR < 1 meant that metformin protected the breast cancer patients from metastasis, which is inversed when HR/RR > 1. Mantel-Haenszel random-effects model was chosen because of the assumption that studies might be different for random errors and between study variability and the pooled HRs/RRs with associated 95% CI were obtained upon generic inverse variance approach, which allowed the weight of each study based on variance and adjusted point estimates and standard errs from individual study to be extracted and combined [22], by Revman 5.3 software (RevMan, The Cochrane Collaboration). The Cochcrane Q test and I^2 statistic were used to assess and quantify heterogeneity, when $I^2 < 25\%$, no significant heterogeneity; 25% − 50%, low heterogeneity; 50% − 75%, medium heterogeneity; > 75%, high heterogeneity [23]. Due to the limitation of the quantity of studies, the egger’s test was appropriate and we meant to use Funnel plot to show the publication bias of the included studies. A two-tailed $p < 0.05$ was thought statistically significant.

Results

Studies included in the meta-analysis

From the 7 databases, we achieved 1121 literatures with search terms. 773 articles were left after deleting the duplication, while 46 articles were eligible for further full text assessment. After carefully reading full text, 8 studies met the inclusion criteria. The details of these studies were shown in Fig. 1.

The characteristics of studies and patients

The 8 studies published before May 2020, included 13919 breast cancer patients, with a maximum sample size of 6769 and a minimum sample size of 61 participants. 4 in China[24–27], 1 in South Korea[28], 1 in America[29], 1 in Germany[30], 1 in Egypt[31]. Moreover, 2 were RCTs while 6 were retrospective cohort studies. Among these studies, 3598(25.73%) patients received metformin adjuvant therapy and 5784(41.56%) patients combined with T2DM. For other features of included studies, check Table 1.

Table 1
Characteristics of included studies

Authors	Year of publication	Country	Study type	NOS score	Sample size	The numder of breast cancer patients with T2DM	Follow-up time(average or median, month)	Age (average or median, range, year)	metformin dose	other treatment than antihyperglycem therapy
Soley Bayraktar	2012	Amercia	retrospective cohort studies	8	1448	130	62	41(21–87)	not mentioned	All received surgery and adjuvant chemotherapy(if necessary)
Tangyan He	2017	China	retrospective cohort studies	7	61	34	44	61.25(42–82)	not mentioned	All received surgery, chemotherapy and hormonal therapy(if necessary)
Louis Jacob	2016	Germany	retrospective cohort studies	7	4953	4953	55.2	71.4(40–90)	not mentioned	not mentioned
Hee Jeong Kim	2015	South Korea	retrospective cohort studies	7	6967	386	100.3	55	not mentioned	Chemotherapy and Hormonal therapy(if necessary)
Weili Min	2020	China	retrospective cohort studies	7	89	89	55.1	64.4(55–73)	not mentioned	surgery
Sahar Mohammed EL-Haggar	2016	Egypt	RCT	not applicable	102	0	22.8	48.8(40–65)	850 mg	All recevievd Chemotherapy and 83.3% Hormonal therapy(if necessary)
Haiyan Wang	2017	China	RCT	not applicable	128	128	55	57.2(45–76)	850 mg	surgery
Wenjie Zhu	2013	China	retrospective cohort studies	6	171	64	45	52.4(24–79)	not mentioned	All received surgery, chemotherapy and hormonal therapy(if necessary)

Metastasis

A total of 8 studies investigated the relationship between adjuvant metformin and distant metastasis of breast cancer but only 3 of them reported that added metformin into general anti-tumor therapy prolonged DMFS with statistically significant. The analysis showed that metformin addition is related with inhibition of metastasis of breast cancer (HR = 0.69, 95% CI = 0.57–0.82, $p < 0.0001$, $I^2 = 0\%$) (Fig. 2A). Because different types of studies were included, we conducted subgroup analysis for RCTs (HR = 0.35, 95% CI = 0.16–0.78, $p = 0.01$, $I^2 = 0\%$) (Fig. 2B) and retrospective cohort studies (HR = 0.71, 95% CI = 0.59–0.86, $p = 0.0003$, $I^2 = 0\%$) (Fig. 2C) respectively.

Considering that breast cancer patients using metformin were likely to be T2DM, we selected patients with breast cancer and T2DM from the included studies for subgroup analysis. Ultimately, 7 studies were eligible while the study population of Sahar's research were all nondiabetic breast cancer women. It turned out that metformin protected T2DM breast cancer patients from metastasis (HR = 0.68, 95% CI = 0.57–0.82, $p < 0.0001$, $I^2 = 0\%$) (Fig. 3A). We performed subgroup analysis for 6 retrospective cohort studies only (HR = 0.69, 95% CI = 0.57–0.83, $p < 0.0001$, $I^2 = 0\%$) (Fig. 3B), for just 1 RCT included patients with T2DM.

Publication bias

The publication bias was evaluated by Egger's test and displayed by Funnel plot. As shown in Fig. 4, since associated-clinic trials was still low in quantity, the publication bias was difficult to avoid completely. The result of Egger's test of this meta-analysis indicated there was no significant publication bias between the 8 literatures ($p = 0.15$), meaning the result was acceptable and believable.

Discussion

Discussion on the meta-analysis

Discovery anti-tumor drugs is a luxurious and time-consuming process, also the percentage of drugs meets the clinic is quite a little. Thus, the development of new functions of existing drugs has become a hot study topic. It is known to all that obesity and T2DM relate with poor prognosis of breast cancer closely. Metformin, the most widely used antihyperglycemic drug to treat T2DM, could maintain weight loss as well [5]. It has been verified that most tumors are sensitive to metformin [6, 8]. Consistently, metformin synergizes with conventional anticancer therapy to kill tumors and repress migration [32]. But the outcomes of clinical studies of metformin in the treatment of breast cancer were not entirely positive [15, 29, 33], the metformin effect in breast cancer treatment was still under discussion. In this study, we intended to estimate the connection between metformin and distant breast cancer distant metastasis. With the forest plot (Figs. 2 and 3), we demonstrated that adjuvant metformin might contribute to suppress breast cancer metastasis.

The adverse effects brought by metformin should not be neglected, while gastrointestinal distress, including transient mild nausea and moderate diarrhea, was the toxicity that people usually met during metformin treatment [31, 34]. There was no grade 3 and 4 treatment-related adverse event (TRAE) reported in the included publications.

This meta-analysis had several limitations. First, the small sample size limited to obtain firm conclusions. Second, the dose of metformin could not adjust to consistent because most of the trails were retrospective and the individual distinctions among the patients were unavoidable. It has been proved that adjuvant metformin therapy repressed HER 2 + breast cancer cells [35, 36] while ER- breast cancer cells resisted to this drug [37]. Thus, the effects of metformin might depend on the molecular types of breast cancer. Here we recommended that subgroups for different hormone receptors status should be designed in the future clinical trials.

The mechanisms of metformin to suppress tumor metastasis

Inhibiting tumor metastasis by metformin was a complex process of multiple pathways, including: AMPK activation, epithelial mesenchymal transition (EMT) inversion, DNA methylation modulation, interfering with TGF-β pathway and tumor microenvironment. Moreover, N-cadherin, vimentin, β-catenin, snail, Rac1 and MMP-2/9 were downregulated while E-cadherin and phosphorylated AMPK increased, which resulted in tighter intercellular connections, weaker migration and movement of tumor cells. Metformin affected insulin-like growth factor (IGF) pathway by repressing IGF-1 receptor and IGF-2 molecule [38]. As shown in Fig. 5, metformin mediated different pathways to prevent metastasis.

Intercellular reaction

AMPK, a widely known metformin effector, was activated in two pathways. One way was that metformin activated liver kinase B1 (LKB1), the upstream of AMPK, and the other pathway was mitochondrial complex I activation decreased ATP/AMP ratio under metformin stimulation, which triggered AMPK activation indirectly [39, 40]. Phosphorylated AMPK functioned as an inhibitor to repress a series of moleculars activation, containing: STAT3, smad-2/3, Akt, ERK, mTOR, PKCγ and Twist. Snail, which was the downstream of ERK and Y-box binding protein-1 (YB-1) [41, 42], a oncogenic transcription/translation factor, and also the direct target gene of metastasis-related gene YAP [43]. This protein regulated E-cad expression with Twist. Additionally, it participated in E-cad promoter hypomethylation modulation with Slug [41]. Metformin induced Snail ubiquitination after LKB1 phosphorylation, which helped Snail's interaction with E3 ligase FBXL14 [44]. Furthermore, metformin decreased Twist with obliterating interaction between GSK-3β and Twist via reducing Akt/GSK-3β pathway [45]. mTOR, of which inhibition under metformin therapy mediated suppression of HIF-1α/VEGF-A and p70s6k [46, 47], was another downstream molecule of Akt. In addition, metformin augmented Foxo3a nuclear localization and protein stabilization to active Foxo3a with IKKβ repression and MDM2 phosphorylation involvement, leading to the level of E-cad increase [42]. For protein kinase Cγ (PKCγ), which was attenuated after AMPK-α1 phosphorylation, it modulated Hs90α activation [48].

The Rac1 and RhoA GTP downstream migratory protein took charge of the migration of cell, thus they were required in cancer migration and metastasis. Metformin downregulates Rac1 in different pathways. Firstly, metformin suppressed FAK/Akt signaling pathway [49] or CXCL12/CXCR4 [50] to decrease downstream factors Rac1 and RhoA GTP expression. Secondly, metformin elevated the level of phosphatase and tensin (PTEN), a protein controls tumor metastasis, to inhibit Akt/Rac1 axis [51]. Thirdly, Rac1 GTP was reduced by metformin-mediated cAMP increase [50]. Moreover, CD24, a mucin-like adhesion molecule, enhanced the metastasis potential of malignant cells. Distant metastasis in patients with refractory breast cancer was mainly composed of CD24 positive cells, which was thought as a marker indicating poor prognosis of breast cancer. Recently, CD24 has been confirmed to significantly be downregulated by metformin [52].

Micro-RNA, modulators of many cellular signaling pathways, have been verified to take parts in metformin treatment. MiR-26a was enhanced under metformin stimulation to inhibit Akt phosphorylation [53]. Similarly, miR-381, upregulated by metformin, significantly interfered with YAP transcription to affect Snail [43]. MiR-30a, another member upregulated by metformin, attenuated SOX4, which was a oncogenic transcription factor and epithelial mesenchymal transformation (EMT) regulator, reversing the process of EMT [54]. Because of the DNA methylation, metformin therapy increased the level of mir-570-3p, while decreased lncRNA H19 by metformin-induced DNA methylation. The former was shown to reduce the invasion of tumor cells through inhibiting LCMR1 and ATG12 [55], while the latter was also been implicated to control tumor metastasis by not only reducing MMP-9, but also elevating AMPK phosphorylation and let-7, a potent tumor suppressor microRNA [56, 57].

Effects on tumor microenvironment cells

The word "tumor microenvironment" was created in 2011, and defined to include endothelial cells, pericytes and immune inflammatory cells [58]. Endothelial cells were shown to benefit to developmental and tumor-associated angiogenesis, while pericytes wrapped around the endothelial tubing of blood vessels and prevented the tumors from entering the circulatory system, making it possible to reduce subsequent hematogenous dissemination [59, 60]. Metformin downregulated micro-vessel density (MVD) by inhibiting platelet-derived growth factor B (PDGF-B), ending with reducing the ratio of endothelial cells/pericytes, leakage and hypoxia, namely "vessel normalization" [61, 62]. Moreover, Angiopoietin-like protein 4 (ANGPIL4) was decreased during metformin

treatment after HIF-1α suppression [63]. Another modulation of metformin to block tumor metastasis was the attenuation of M2-like polarization of tumor associated macrophages (TAM) with phosphorylated AMPK α1 [64].

Conclusion

To the best of our knowledge, this was the first meta-analysis focused on the relationship between metformin and distant metastasis of breast cancer. The study showed that metastasis repression could be achieved by receiving metformin and general breast cancer treatment combination, and the association was supported by a wide range of basic studies, implicating the possibility for metformin becoming a new anticancer drug. However, this report was lack of prospective research, therefore, the veracity of conclusion remained to be verified. Further studies investigating connection metastasis of breast cancer and metformin were expected.

Abbreviations

CNKI
China National Knowledge Infrastructure; RCT:randomized controlled trial; NOS:Newcastle-Ottawa Scale; HR:hazard ratio; RR:risk ratio; CI:confidence interval; DMFS:distant metastasis-free survival; T2DM:type 2 diabetes mellitus; BCSC:breast cancer stem cell; AJCC:American joint committee on cancer; ER:estrogen receptor; TRAE:treatment-related adverse event; IGF:insulin-like growth factor; AMPK:adenosine 5'-monophosphate-ativatied protein kinase; LKB1:liver kinase B1; YB-1:Y-box binding protein-1; PKCγ:protein kinase Cγ; PTEN:phosphatase and tensin; EMT:epithelial mesenchymal transformation; TGF-β:transforming growth factor; ERK:extracellular signal regulated kinase; STAT:Signal Transducer and Activator of Transcription; Akt:protein kinase B; IKKβ:inhibitor kappa B kinase β; CXCL:C-X-C motif ligand; CXCR:C-X-C motif receptor; cAMP:cyclic adenosine moriophosphate; PDGF-B:platelet-derived growth factor B; MVD:micro-vessel density; ANGPIL4:Angiopoietin-like protein 4; VEGF:vascular epidermal growth factor; TAM:tumor associated macrophages.

Declarations

Competing interests

The authors declare that there are no conflicts of interest.

Availability of data and materials

The datasets supporting the conclusions of this study are included within the article and its additional files.

Authors' contributions

YYW and ZHZ screened the reference. YYW drafted the manuscript and analyzed most of the data. YYW,JYP,XX and XW discussed and revised themanuscript. All authors read and approved final manuscript. Thanks to all authors for their time and effort and department of breast surgery from the first hospital of Jilin University for technical support. All authors have read and approved the manuscript.

Consent for publication

All coauthors were offered the opportunity to read the final manuscript and agreed to publish.

Ethics approval and consent to participate

Not applicable.

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Figures

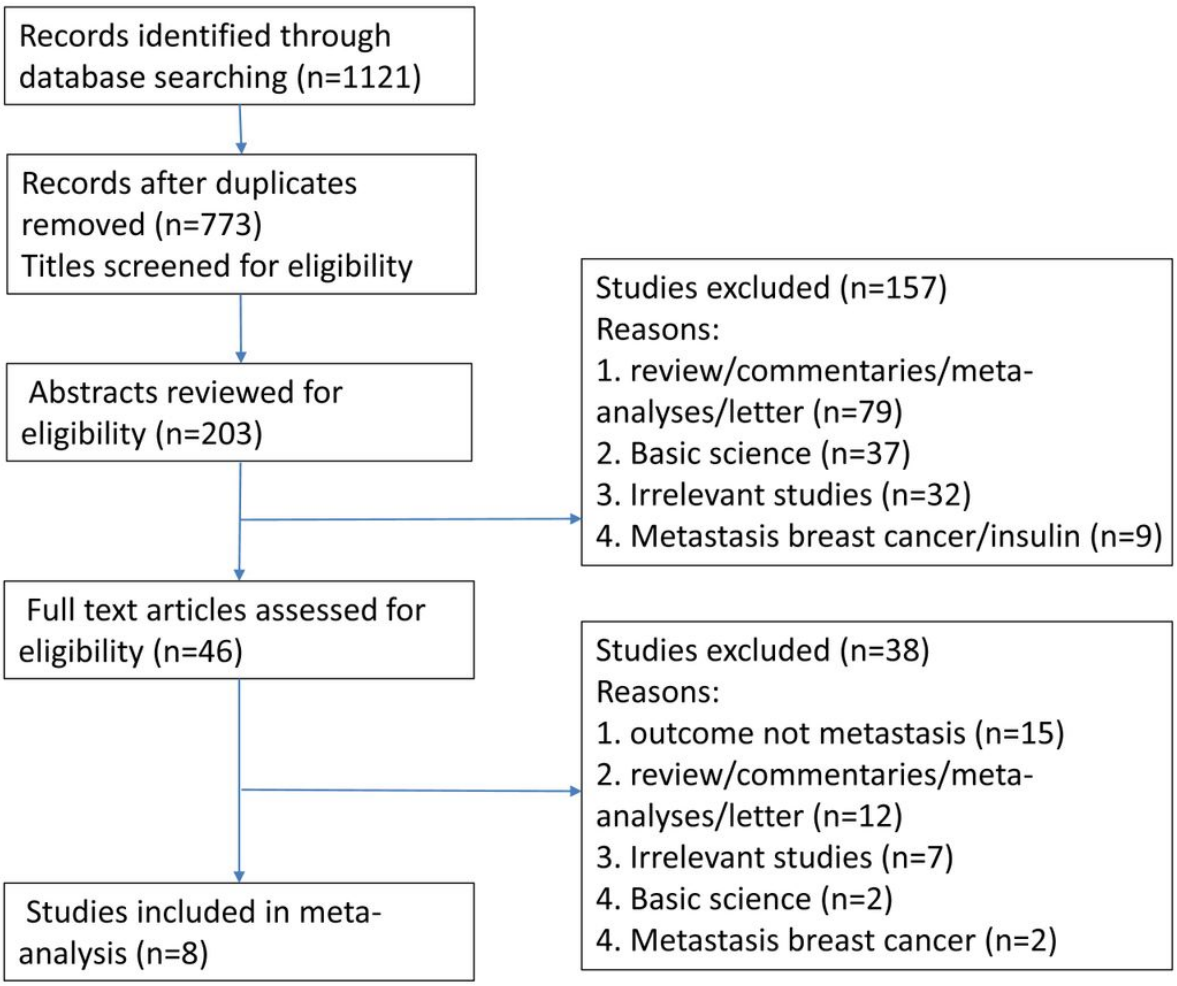

Figure 1

Study selection. Note. n: number

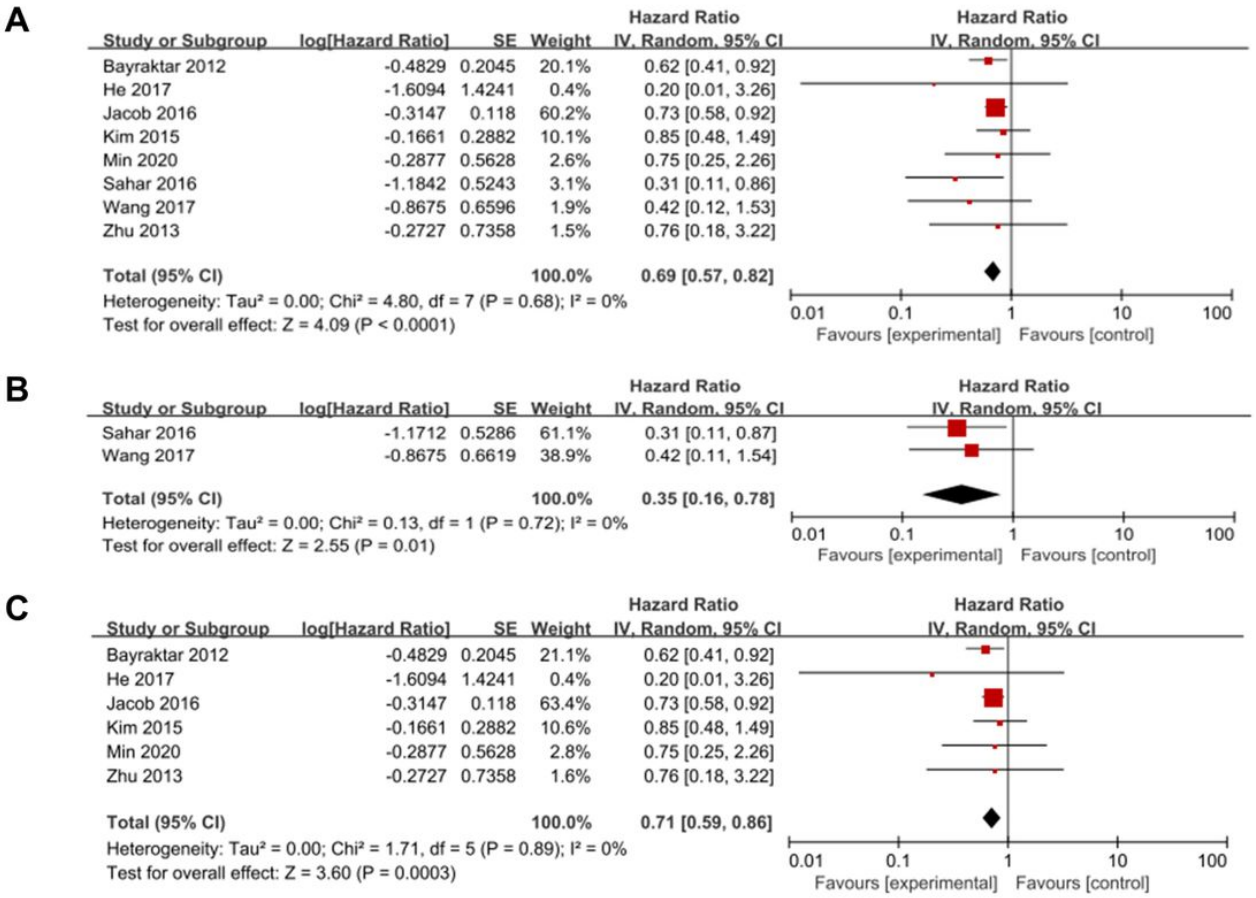

Figure 2

Summary of pooled risk estimates of all breast cancer patients. The association between metformin and general anti-tumor therapy combination used in women with breast cancer and (A) All-type of studies, (B) subgroup of RCTs and (C) subgroup of retrospective cohort studies. Note. HR: hazard ratio, CI: confidence intervals, SE: standard error

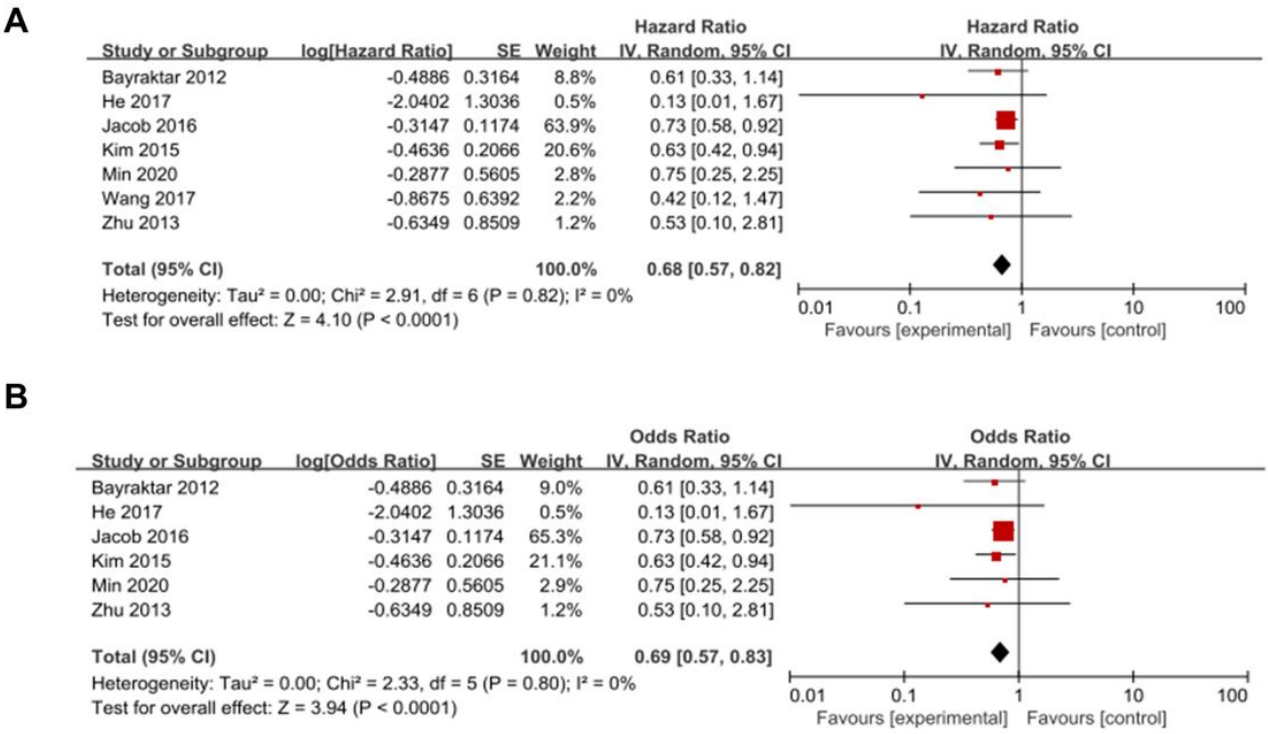

Figure 3

Summary of pooled risk estimates of breast cancer patients with T2DM. The association between metformin and general anti-tumor therapy combination used in women with breast cancer and T2DM. (A) All-type of studies, (B) subgroup of retrospective cohort studies. Note. HR: hazard ratio, CI: confidence intervals, SE: standard error

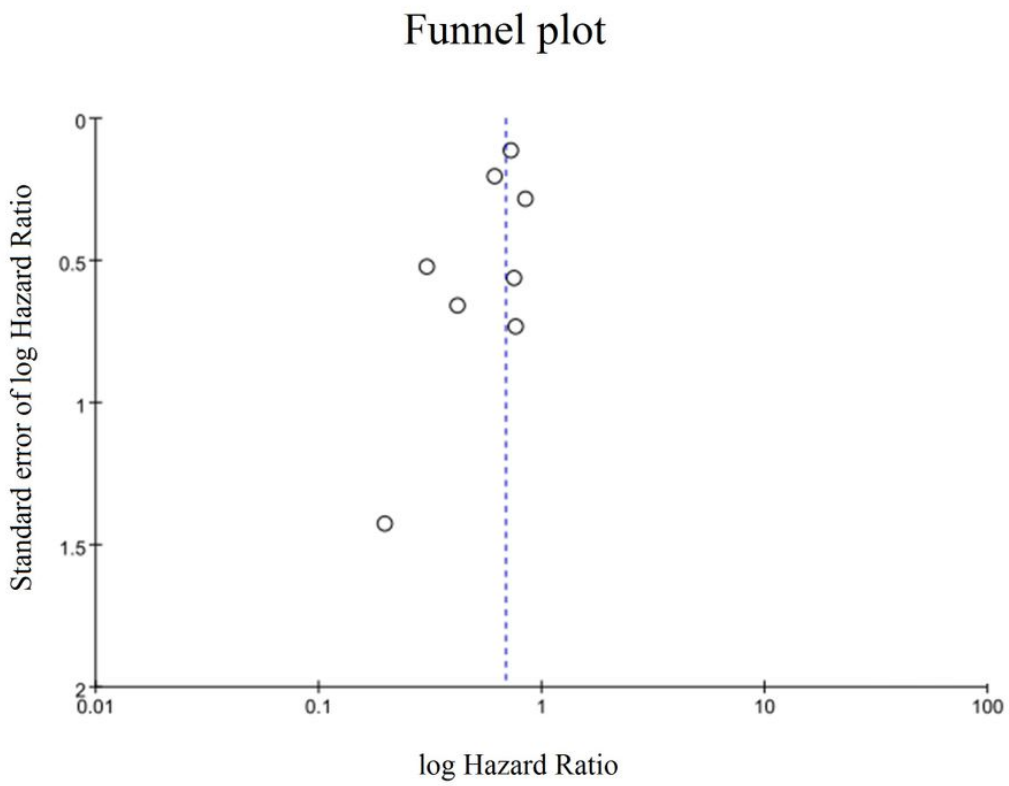

Figure 4

Funnel plot about publication of included studies.

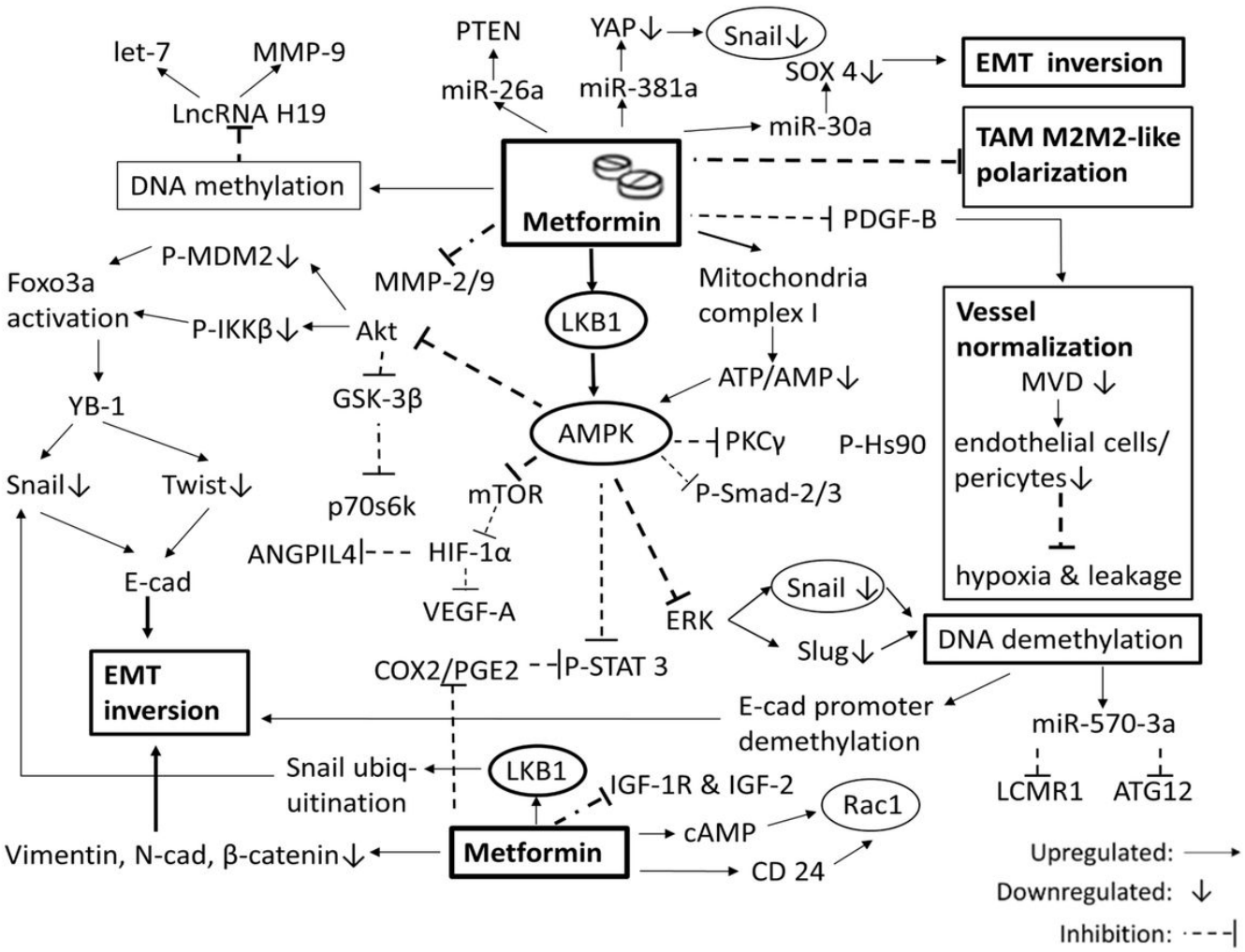

Figure 5

The possible mechanisms for metformin to suppressed tumor metastasis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- metforminNOS.xlsx
- Riskofbiasgraph.pdf
- MetforminPRISMA2009checklist.doc