Role of von Willebrand factor in the angiogenesis of lung adenocarcinoma (Review)

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Abstract. Lung adenocarcinoma (LUAD) has a high morbidity and mortality rate worldwide, and its growth and metastasis require angiogenesis. The density of microvessels in LUAD is positively correlated with metastasis and recurrence. Von Willebrand factor (VWF) is a multifunctional glycoprotein in blood plasma. Recent evidence shows that VWF inhibits angiogenesis through regulation of angiopoietin-2 (Ang-2) and integrin αvβ3. LUAD patients exhibit an increase in the plasma VWF/ADAMTS-13 ratio. Gene expression profiles of LUAD tissues indicate that *VWF* is differentially expressed in LUAD tissues compared to normal tissues. GATA binding protein 3 (GATA3) transcription factor may mediate *VWF* expression in LUAD. In this review, we summarize the role of VWF in LUAD and its regulatory mechanisms. We also discuss the potential of VWF as a diagnostic indicator and therapeutic target of LUAD.

Contents

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Abbreviations: LUAD, lung adenocarcinoma; VWF, von Willebrand factor; Ang-2, angiopoietin-2; ECs, endothelial cells; NSCLC, non-small cell lung cancer; VEGF, vascular endothelial growth factor; VEGFR-2, endothelial growth factor receptor-2; LUSC, lung squamous cell carcinoma; VWD, von Willebrand disease; WPBs, Weibel-Palade bodies; ERG, ETS-related gene; Cd, cadmium; OCT, octamer-binding protein; NFI, nuclear factor-I; NF-κB, nuclear factor-κB; NFY, nuclear factor Y; dDAVP, desmopressin; r VWF, recombinant VWF; YYI, Yin Yang 1

Key words: von Willebrand factor, angiogenesis, lung adenocarcinoma, endothelial cells

1. Introduction

Lung adenocarcinoma (LUAD) is the most common subtype of non-small cell lung cancer (NSCLC), which accounts for approximately 40% of all lung cancer (1). The two most common NSCLC histologic types are LUAD and lung squamous cell carcinoma (LUSC) (2). LUAD cells develop from small airway epithelial cells and the most distal epithelial cells of the lung (3). From adenocarcinoma *in situ* to minimally invasive adenocarcinoma to overt invasive adenocarcinoma, LUAD progresses in stages (4). In addition, LUAD cells may easily invade the walls of blood vessels and lymphatic vessels and thus metastasize, resulting in poor patient prognosis (5). Although surgical resection, radiation therapy and immunotherapy have made great progress in recent years, the 5-year relative overall survival rate of LUAD patients is approximately 18% (6).

Angiogenesis occurs mainly at the expanding borders of tumor cells in primary LUAD in a hypoxic environment (7). In hypoxic conditions, tumor cells produce and secrete pro-angiogenic cytokines, such as vascular endothelial growth factor (VEGF), which activate endothelial cells (ECs) (8). Coincidentally, proliferative ECs have been observed near the alveolar microvasculature. Furthermore, both ECs and tumor cells secrete matrix metalloproteinases (MMPs), which degrade the extracellular matrix (ECM) and basement membranes. Primary sprouts form tubes and then capillary loops, which are followed by pericyte recruitment, synthesis of a new basement membrane, and vessel maturation (9). Low dose of cadmium (Cd) may promote angiogenesis through upregulation of VEGF expression and secretion and promote the development of LUAD (10). In addition, reducing VEGF signaling may effectively inhibit the development of LUAD (11).

Anti-angiogenic drugs that inhibit VEGF signaling pathways, such as ramucirumab and bevacizumab, have been considered a promising option for patients with advanced NSCLC (including LUAD) (12). However, some side effects such as proteinuria, hypertension, and hand and foot

syndrome often accompany the treatment with angiogenesis inhibitors that include sorafenib, bevacizumab, and ramucirumab (13,14).

Recently, Starke *et al* (15) demonstrated that von Willebrand factor (VWF) regulates angiogenesis. The VWF is a component of hemostasis, promoting the binding of platelets and ECs at the site of vascular injury. VWF recruits and tethers platelets at sites of vascular injury, facilitating platelet aggregation (16). In addition, VWF acts as a protective carrier molecule for procoagulant factor VIII (FVIII). Thrombotic thrombocytopenic purpura (TTP), a deadly disease characterized by widespread deposition of VWF and platelet-rich thrombi in the microvasculature (17), is caused by a lack of VWF-specific metalloprotease ADAMTS-13 [a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; also known as VWF-cleaving protease (VWFCP)] (18). von Willebrand disease (VWD) is caused by lack of VWF. Knockdown of *VWF* expression in ECs leads to increased migration and proliferation in response to VEGF (15). This may be consistent with the clinical observation that vascular malformations can cause angiodysplasia in certain patients with VWD. VWF may reduce the migration and proliferation of VEGFR-2-dependent ECs by inhibiting angiopoietin-2 (Ang-2) release and increasing integrin αvβ3 (15).

2. VWF

Structure of VWF. The domains of VWF are ordered symmetrically as follows: D1-D2-D'D3-A1-A2-A3-D4-C1-C2-C3-C4-C5-C6-CK (19) (Fig. 1). The D regions are divided into smaller lobes or modules: the D3, D2, and D1 domains are divided into E, TIL, C8, and VWD modules, respectively (19). D', on the other hand, only has the subdomains TIL' and E (19).

Function of VWF. By tethering platelets to areas of endothelial injury and acting as a carrier for coagulation factor VIII, VWF promotes hemostasis. In addition, other functions of VWF have been identified, including immune response (20), tumor metastasis (21), and leukocyte recruitment (22). Recent evidence suggests the potential clinical detection value and potential prognostic value of plasma VWF in patients with acute myocardial infarction (23,24), type 2 diabetes mellitus with cardiovascular complications (25) and coronary artery disease with major adverse cardiovascular events (26). In addition, the results of *in vivo* and *in vitro* research suggest that VWF controls angiogenesis and that deficiency of VWF leads to increased angiogenesis (27).

Plasma VWF levels are higher in patients presenting with several types of cancer (28,29). Elevated VWF remains an independent predictor of venous thrombosis in cancer patients after adjusting for patient-related factors (30,31). Higher VWF levels in cancer patients are associated with cancer progression and metastasis (21). Endothelial secretion of VWF contributes to the adhesion and transendothelial migration of breast cancer cells (32). Furthermore, new evidence reveals that VWF regulates tumor cell proliferation and apoptosis (32).

3. Expression of VWF in LUAD

Plasma VWF and VWF/ADAMTS-13 ratios were found to be significantly increased in patients with advanced NSCLC (including LUAD and LUSC), while the levels of ADAMTS-13 were decreased (28). ADAMTS-13 cleaves VWF in blood in the A2 domain. Furthermore, a marked increase in the VWF/ADAMTS-13 ratio is associated with fibrinogen, D-dimers and coagulation factor VIII (28). ECs of certain microvessels and small vessels in the lung express abundant *VWF* mRNA. The alveolar-capillary ECs do not express VWF (33). Conversely, other vessels, including the larger vessels, arterioles and bronchial capillaries in the lung, consistently express VWF (33). Xu *et al* (34) discovered that VWF was overexpressed in tumor vessels of LUAD compared to vessels of adjacent tissues. Consistently, VWF expression was found to be elevated in ECs of transplanted mouse LUAD tissues and fresh human LUAD tissues (34). Similarly, Jin *et al* (33) discovered that VWF expression is elevated in normal alveolar-capillary ECs near areas of EC germination and tumor invasion. Meanwhile, the cytoplasm of capillary ECs was enlarged and had increased Weibel-Palade bodies (WPBs), which contain VWF, Ang-2, and other angiogenesis mediators (33). However, alveolar-capillary ECs in LUAD developed new reactivity to VWF (35). The Cancer Genome Atlas (TCGA) and The Gene Expression Omnibus (GEO) dataset GSE43458 were used to explore differentially co-expressed genes between LUAD and normal tissues (36). The *VWF* expression was downregulated in LUAD compared to normal tissue (36).

4. Transcription factors regulate gene expression of *VWF*

VWF expression is restricted to ECs and macrophages. The *VWF* gene sits on the short arm of chromosome 12, spanning ~178 kb. The transcription factors GATA3, ERG, and YY1 have been shown to act as regulators of *VWF* transcription (37,38) (Fig. 2).

ETS-related gene. The ETS-related gene (ERG) is an ETS family transcription factor specifically expressed in ECs (39), and regulates a series of EC-specific genes (40). By binding with the -56 ETS motif of the *VWF* promoter, ERG maintains basal expression of VWF (37). In addition, ERG mediates cadmium (Cd)-mediated VWF expression, suggesting that ERG is involved in the transcriptional control of *VWF* in pathological situations (41). However, the protein and mRNA levels of ERG were unchanged with A549-derived conditioned medium (CM) (34).

GATA3. GATA protein 3 (GATA3) is a transcription factor that belongs to the zinc finger protein family and can recognize (A/T)GATA(A/G) and related sequences. *In vivo* research has shown that loss of GATA-1 expression in megakaryocytes causes decreased levels of *VWF* mRNA (42). A GATA-binding motif can be found in the *VWF* promoter at position +220 (38). Furthermore, GATA3 expression was found to be increased in A549-CM co-cultured human umbilical vein ECs by binding GATA3 to the +220 GATA binding motif in the *VWF* promoter (34). Therefore, GATA3 may upregulate VWF expression in LUAD (34).

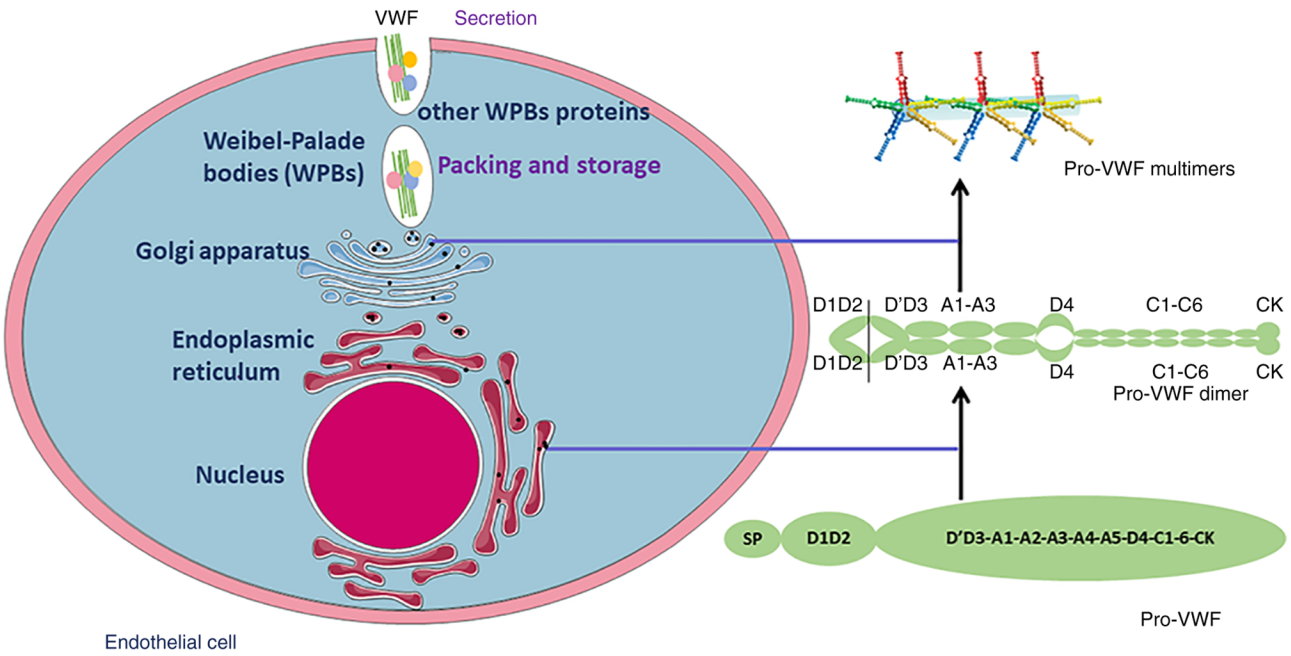

Figure 1. Schematic diagram of VWF synthesis and secretion by ECs and its structure. Alignment of VWF structural domains: D1-D2-D'D3-A1-A2-A3-D4-C1-C2-C3-C4-C5-C6-CK. In the endoplasmic reticulum, VWF is a dimer through disulfide bonds. Thereafter, this dimer is transported to the Golgi, where multimerization occurs. Subsequently, VWF multimers form tubules and are stored in the WPBs. WPB exocytosis underlies hormone-evoked VWF secretion from ECs. VWF, von Willebrand factor; ECs, endothelial cells; WPBs, Weibel-Palade bodies.

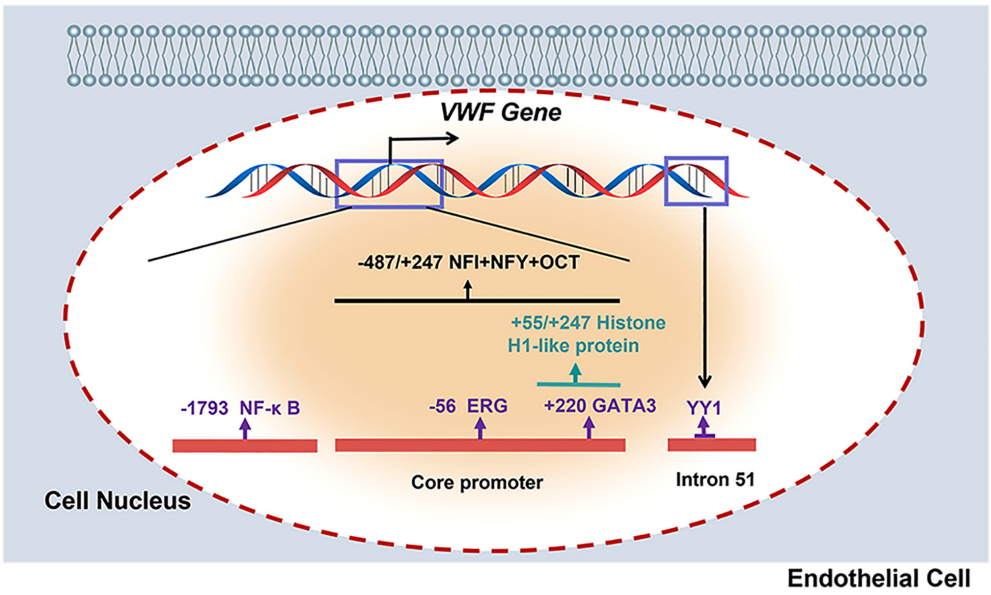

Figure 2. Regulatory elements on the *VWF* gene. ERG sustains *VWF* basal expression by binding to the -56 ETS region of the *VWF* promoter. Furthermore, the +220 GATA binding motif in the *VWF* promoter is where GATA3 binds. A region of intron 51 of the *VWF* gene interacts with a specific complex containing YY1. Furthermore, the NF-κB binding site on the *VWF* promoter at position-1793 suppresses *VWF* transcription. *In vivo* regulation of the *VWF* promoter sequence-487/+247 by OCT, NFI, and NFY occurs. In addition, histone H1-like proteins repress *VWF* transcription by binding to the *VWF* promoter sequence +55/+247. VWF, von Willebrand factor; ERG, ETS-related gene; GATA3, GATA binding protein 3; YYI, Yin Yang 1; OCT, octamer-binding protein; NFI, nuclear factor-I; NF-κB, nuclear factor-κB; NFY, nuclear factor Y.

Yin Yang 1. Yin Yang 1 (YY1) is a ubiquitous transcription factor that has both activating and repressive effects. The AATGG sequence is shown to the core consensus binding site of transcription factor YY1 (43). A region in intron 51 of the *VWF* gene is DNase I hypersensitive (HSS)-specific in non-endothelial cells and interacts with a specific complex of endothelial and non-endothelial cells containing YY1 (43). In addition, the HSS sequence of intron 51 of the *VWF* gene contains a *cis*-acting element that is required for *VWF* gene transcription in a subpopulation of lung ECs (43).

In addition, other transcription factors regulate *VWF* promoter activity (Fig. 2). Octamer-binding protein (OCT) and nuclear factor-I (NFI) inhibits *VWF* promoter activity, whereas histone H1-like protein increases promoter

activity (44). The nuclear factor (NF)-κB binding site located at -1793 of the *VWF* promoter inhibits *VWF* transcription (45). In addition, *trans*-acting factor nuclear factor Y (NFY) has been confirmed to be both a repressor and an activator of the *VWF* promoter (44).

5. Synthesis and secretion of VWF

The biosynthesis of VWF includes several posttranslational modifications in ECs and megakaryocytes (46). The polypeptide of VWF contains 741 characteristic amino acid residues, 22 amino acid long signal peptides, and 2,050 amino acid residue long mature polypeptides (46). In the endoplasmic reticulum, VWF is a dimer (called pro-VWF) through disulfide bonds. Thereafter, this dimer is transported to the Golgi, and it is multimerized through a disulfide bond between the D'D3 structural domains. Subsequently, VWF multimers form tubules and are stored in Weibel-Palade bodies (WPBs) (47).

VWF is secreted through two main pathways. One is regulatory and responds to secretion, and the other is continuous and does not require cellular stimulation (46). Secretion of VWF from specialized storage granules, called WPBs, is triggered by several substances (48). When WPBs are stimulated by a variety of substances such as histamine, thrombin, and phorbol myristate acetate, they release amounts of ultra-large VWF. Once released, they are cleaved in the A2 domain by ADAMTS-13. Thus, VWF circulates in plasma in the form of a series of multimers ranging in size from 500 to 20,000 kDa (42). Plasma VWF is almost exclusively derived from endothelial secretion, and VWF secreted into the subendothelium has a role in EC adhesion and extracellular matrix binding (49).

6. VWF regulates angiogenesis of LUAD

Inhibition of *VWF* expression in ECs with short interfering RNA leads to increased angiogenesis *in vitro*, increased VEGFR-2-dependent migration and proliferation, along with increased Ang-2 release and decreased integrin αvβ3 levels (15). VWF may negatively regulate VEGF-dependent angiogenesis through pathways involving Ang-2 and integrin αvβ3 (50).

Integrin αvβ3. The VWF may regulate angiogenesis and vascular homeostasis by binding integrin αvβ3 (51). Under certain conditions, integrin αvβ3 can inhibit VEGFR-2 activity and downstream signaling to suppress angiogenesis (52). The absence of *VWF* in ECs leads to integrin αvβ3 expression decrease, which may cause VEGFR-2 signaling increase (53). Interestingly, VWF also interacts with integrin αvβ3 on vascular smooth muscle cells via the Notch signaling pathway (54). However, pharmacological inhibition of integrin αvβ3 inhibits blood vessel generation in experimental models (55). Thus, integrin αvβ3 may have a bimodal effect in regards to angiogenesis, which acts as an activator or an inhibitor depending on the stage of angiogenesis and the different extracellular matrix ligands.

Weibel-Palade body proteins: Angiopoietin-2. VWF may promote the formation of WPBs, which contain angiopoietin-2 (Ang-2) and VWF (56). Reduced or dysfunctional VWF leads to a reduction in WPBs, resulting in the component release of WPB components such as Ang-2 (56). Barton *et al* found an increase in Ang-2 in VWF-deficient ECs *in vitro* (57). This has now been confirmed *in vivo*, with a significant increase in Ang-2 levels in the brains of *Vwf* $^{-/-}$ mice (58). In addition, the binding of Ang-2 to its receptor Tie-2 can act synergistically with VEGFR-2 signaling to promote angiogenesis (59). Excessive and dysregulated VEGF signaling can lead to the formation of fragile and leaky blood vessels (60). For example, patients with VWD show a high prevalence of gastrointestinal vascular malformations.

In addition to integrin αvβ3 and Ang-2, VWF also interacts with galectin-3 (61), galectin-1, and insulin-like growth factor binding protein-7 (62). Meanwhile, the interaction of VWF with GPIba has been reported to affect cell migration.

The angiogenic factors VEGF and fibroblast growth factor-2 (FGF-2), which are abundant in the tumor microenvironment, have been shown to upregulate VWF expression. Treatment with bevacizumab, an anti-VEGF, has been demonstrated to lower VWF levels in the blood (63). *In vitro*, VWF binds to VEGF-A through the heparin-binding domain (HBD) within the VWF A1 domain (64). Incorporation of the A1-HBD domain of VWF protein into fibrin matrices enables sequestration and slows release of incorporated VEGF-A (64).

7. Discussion

Plasma VWF and the VWF/ADAMTS-13 ratio have been found to be substantially increased, whereas ADAMTS-13 levels were found to be decreased in patients with advanced NSCLC (28). Tumor cells directly induce activation of ECs, leading to WPB extravasation and release of ultra-large VWF multimers (65). Ultra-large VWF multimers are discharged into the plasma, where the plasma VWF-cleaving protease ADAMTS-13 rapidly degrades them into smaller VWF multimers (66). Smaller VWF multimers are more rapidly cleared from the circulation than ultra-large VWF (67). Increased ultra-large VWF in plasma disrupts the balance between VWF and ADAMTS-13 levels, resulting in an increased VWF/ADAMTS-13 ratio (68). The VWF/ADAMTS-13 ratio has been used to diagnose hypercoagulability caused by an imbalance in VWF secretion and ADAMTS-13 in patients with organ failure (69). In patients with advanced NSCLC, a marked increase in the VWF/ADAMTS-13 ratio was found to be positively correlated with D-dimers, fibrinogen and coagulation factor VIII (28). Therefore, elevated VWF/ADAMTS-13 levels implicate a highly thrombotic state, resulting in thrombosis in cancer patients. VWF/ADAMTS-13 in plasma has the potential to be used as a marker of prognosis in patients with LUAD.

VWF was found to be preferentially overexpressed in tumor vessels of LUAD compared to vessels of adjacent tissues (34). Consistently, overexpression of VWF was found in ECs of transplanted mouse LUAD tissues and fresh human LUAD tissues (34). However, VWF was recently found to be expressed in normal lung tissue, but low or undetectable levels were found in LUAD tissue (36). Furthermore, survival

analysis showed that LUAD patients with low VWF expression in tissues had a poorer prognosis (70). Thus, VWF may be differentially expressed in different stages of LUAD. The association between VWF levels and LUAD staging may be explored and potentially used for prognosis.

The mechanism of VWF regulation of tumor angiogenesis in LUAD has not been elucidated. VWF may act as a negative regulator of VEGF-dependent angiogenesis through pathways involving integrin αvβ3 and Ang-2 (50). In addition to integrin αvβ3 and Ang-2, VWF interacts with galectin-3 and galectin-1, which are involved in the control of angiogenesis. Supplementation with VWF analogs may inhibit tumor angiogenesis in LUAD. There are several medications available to elevate VWF with no significant side effects. Desmopressin (dDAVP) is a treatment for patients with VWD and stimulates the release of endogenous VWF into the plasma (71). MINIRIN® (dDAVP) is supplied by Ferring/Valeas (71). The recommended dosage is 0.3 μg/kg by slow i.v. infusion or fixed doses of 150 μg in children and 300 μg in adults by intranasal spray (71). A human recombinant VWF (rVWF), vonicog alfa, was found to increase VWF levels in VWD patients, making treatment independent of plasma supply (72). rVWF is a purified glycoprotein synthesized in a genetically engineered CHO cell line (72). The doses of 50 and 80 U/kg VWF have been used for evaluation (72). These drugs may treat LUAD by increasing VWF in the blood to inhibit tumor angiogenesis. Paradoxically, VWF has the potential to promote tumor metastasis (21). Tumor cells of nonendothelial origin may acquire *de novo* VWF expression and show enhanced EC adhesion and extravasation (21). In addition, tumor cells directly induce EC activation resulting in WPB exocytosis and the release of ultra-large VWF strings (21). VWF binds to platelets via GPIbα and GPIIb/IIIa receptors and to tumor cells via GPIIb/IIIa receptors or their semi-homologous twin integrin αvβ3 (65), and, therefore, may tether platelets and tumor cells along the endothelium (21). This interaction may increase tumor cell adhesion to the vascular endothelium and promote extravasation (21). The balance of the potential benefits and risks of VWF treatment on LUAD should be carefully considered. Given that VWF inhibits angiogenesis and thus LUAD growth, VWF supplementation may achieve therapeutic effects in LUAD. However, VWF may also promote tumor metastasis. VWF supplementation is not recommended for patients with early-stage LUAD to avoid the risk of tumor metastasis. Anti-angiogenesis therapy is essential for patients with advanced LUAD, thus VWF supplementation may be attempted together with conventional chemotherapy.

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Availability of data and materials

Not applicable.

Authors' contributions

ZL conceived and designed the review. XL wrote the first draft. XL and ZL participated in writing of the manuscript. All authors contributed to the article and read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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