Review Article

Anti-angiogenic therapy in ovarian cancer: current situation & prospects

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Ovarian cancer (OC) is one of five leading causes of cancer related death among women worldwide. Although treatment has been improving, the survival rate has barely improved over the past 30 years. The fatality rate is due to asymptomatic early signs and the lack of long-term effective treatment strategies for advanced disease. Angiogenesis is an important process in tumour growth and metastasis and is the creation of new blood vessels from existing blood vessels. It is a dynamic and complex process involving various molecular regulatory pathways and multiple mechanisms. The inhibition of angiogenesis has become a recognized therapeutic strategy for many solid tumours. While benefits in progression-free survival have been observed, the OS is far from satisfactory for OC patients who receive antiangiogenic therapy. In this article, the present research status of angiogenesis in OC was reviewed and the reasons for poor antiangiogenic therapeutic effects was explored with the aim to identify potential therapeutic targets that may improve the effect of antiangiogenic therapies.

Key words Angiogenesis - antiangiogenic therapy - bevacizumab - ovarian cancer - therapeutic targets

Ovarian cancer (OC) is the most lethal of gynaecological malignancies, and high-grade serous ovarian carcinoma (HGSOC) has the highest degree of malignancies[1]. Although the methods of surgery and chemotherapy have improved in the recent decades, the progression-free survival (PFS) of OC is only 16 to 22 months, and the five-year survival rate is less than 30 per cent[2]. Furthermore, 60 to 80 per cent of patients with OC can achieve complete remission after surgery and first-line chemotherapy, but 80 per cent of these patients will eventually die due to drug resistance or recurrence[3,4]. Because of the poor prognosis and limited treatment options, researchers are constantly looking for new ways to treat advanced OC. Angiogenesis, the process of generating new capillaries from existing blood vessels, is the key to the growth and metastasis of many solid tumours, including OC[5]. During the process of rapid growth and metastasis, tumour cells continuously secrete many related factors that promote angiogenesis, such that new vascular networks are continuously generated in the tumour tissues for the rapid proliferation of tumour cells[6]. If there is no blood supply, tumours cannot grow to more than 1~2 mm[7]. Therefore, tumour blood vessels are an important target for tumour treatment[8,9].
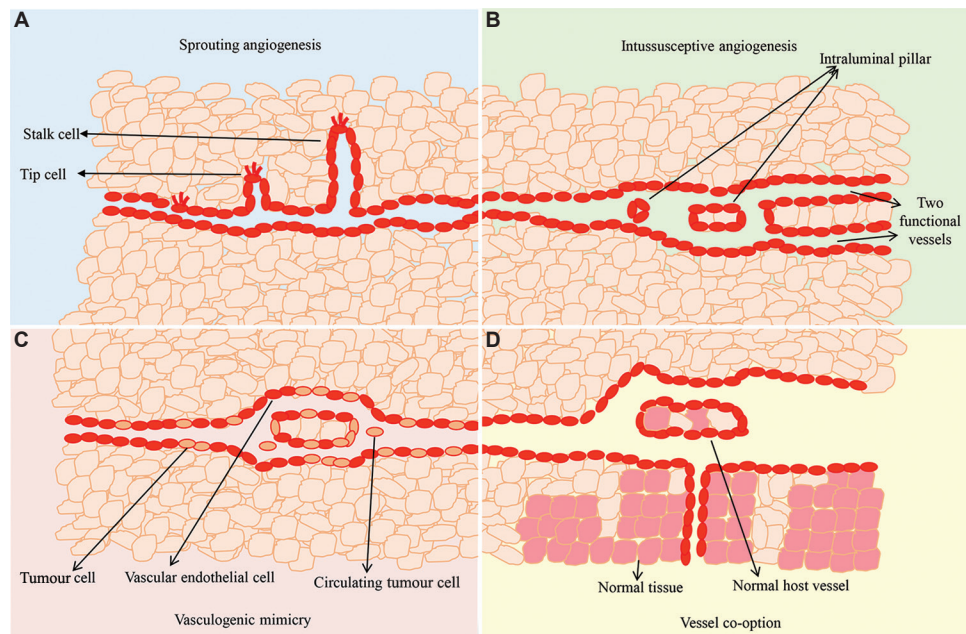
Fig. 1. Tumour neovascularization. (**A**) Sprouting angiogenesis: new blood vessels are produced from an existing blood vessel and are elongated. (**B**) Intussusceptive angiogenesis: insertion of interstitial tissue pillars into the lumen of pre-existing vessels to split it into two new functional vessels. (**C**) Vessel cooption: tumour cells obtain their blood supply by hijacking and moving along the pre-existing vasculature of the host organ. (**D**) Vasculogenic mimicry: tumour cells simultaneously express endothelial and tumour cell markers to form a channel structure for blood perfusion. *Source*: Refs 6,11,12,15,16,17,20.

Pathways of vessel formation in OC

Four main means of a tumour constructing new vessels are shown in Figure 1.

Sprouting angiogenesis: The most common form of angiogenesis in the development of most cancers, including OC, is when new blood vessels are produced from an existing blood vessel and are elongated. In the process of neovascularization, there are two basic cell types involved, the tip and the stalk cells. Tip cells are located at the front of blood vessels and stimulate angiogenesis in the microenvironment through their motile filopodia, while the stalk cells align right behind the tip cells and proliferate speedily, by which the sprouting branch is elongated and the process of lumenization begins[6]. The differentiation of both these cell types is mainly controlled by the vascular endothelial growth factor A (VEGFA) and Notch signalling pathways[10].

Intussusceptive angiogenesis: A typical feature of this type of angiogenesis is the formation of intraluminal pillars. The intussusceptive process is characterized by the insertion of interstitial tissue pillars into the lumen of existing vessels to divide the pre-existing vessel into two new functional vessels[11]. Intussusceptive angiogenesis is significantly better than angiogenic sprouting. Neovascularization is faster and has less metabolic requirements than those generated by angiogenic sprouting because these are independent of EC proliferation, membrane degradation and tissue invasion. Tumours use this strategy to rapidly adapt to changing environments[12]. Intussusceptive angiogenesis has been found in many tumours, such as intestinal cancer and melanoma[13,14]. However, to date, the underlying molecular mechanisms of this process remains unclear.

Vessel cooption: Vessel co-option is a mechanism through which tumour cells obtain blood supply by hijacking and moving along the pre-existing vascular system of host organs. This mechanism often occurs in organs with abundant blood supply, such as the brain and liver[15-17].

Vasculogenic mimicry: Vasculogenic mimicry (VM) refers to the behaviour of tumour cells, which is similar to that of endothelial cells; tumour cells simultaneously express endothelial and tumour cell markers to form a channel structure for blood perfusion. In VM, tumour cells express markers of vascular endothelial cells, such as VE-cadherin[18,19], which play an important role in maintaining pipeline integrity by establishing connections between cells[20]. In addition, tumour cells can be directly integrated into the tumour-associated

Table I. Phase III studies using combination bevacizumab chemotherapy in women with ovarian cancer

Study	Agent	n	Setting	Treatment arm	Clinical outcomes (media PFS, media OS, months)
GOG-218[32]	Bevacizumab	1873	Front-line and maintenance	Paclitaxel+carboplatin+placebo; placebo maintenance versus paclitaxel+carboplatin+bevacizumab; placebo maintenance versus paclitaxel+carboplatin+bevacizumab; bevacizumab maintenance	PFS: 10.3 versus 11.2 versus 14.1 (HR, 0.908; P=0.16)[1] (HR, 0.717; P<0.001)[2] OS: 39.3 versus 38.7 versus 39.7 (HR, 1.036; P=0.76)[1] (HR, 0.915; P=0.45)[2]
ICON-7[33]	Bevacizumab	1528	Front-line and maintenance	Paclitaxel+carboplatin versus paclitaxel+carboplatin+bevacizumab; bevacizumab maintenance	PFS: 17.4 versus 19.8 (HR: 0.87; P: 0.04) OS: 58.6 versus 58.0 (HR: -; P: -)
AURELIA[34]	Bevacizumab	361	Recurrent, platinum-resistant	Chemotherapy (paclitaxel–weekly, topotecan–daily ×5 or weekly, PLD) versus chemotherapy+bevacizumab	PFS: 3.4 versus 6.7 (HR: 0.48; P<0.001) OS: 13.3 versus 16.6 (HR, 0.85; P=0.174)
OCEANS[35]	Bevacizumab	484	Recurrent, platinum-sensitive	Gemcitabine+carboplatin+placebo (combination and maintenance) versus gemcitabine+carboplatin+bevacizumab (combination and maintenance)	PFS: 8.4 versus 12.4 (HR=0.484; P<0.0001) OS: 33.6 versus 32.9 (HR=0.96, P=0.736)
GOG-213[36]	Bevacizumab	674	Recurrent, platinum-sensitive	Paclitaxel+carboplatin versus paclitaxel+carboplatin+bevacizumab; bevacizumab maintenance	PFS: 10.4 versus 13.4 (HR=0.61; P<0.0001) OS: 37.3 versus 42.2 (HR=0.829; P=0.056)

Source: Adapted with permission from ref 31. [1]Paclitaxel+carboplatin+placebo; placebo maintenance versus paclitaxel+carboplatin+bevacizumab; placebo maintenance, [2]Paclitaxel+carboplatin+placebo; placebo maintenance versus paclitaxel+carboplatin+bevacizumab; bevacizumab maintenance. PFS, progression-free survival; OS, overall survival; HR, hazard ratios

blood vessels, which allows the tumour cells to be directly exposed to the bloodstream, leading to tumour metastasis; this process has been reported in a variety of malignant tumour types, including breast, ovarian, prostate, bladder and lung cancers, as well as sarcomas and gliomas[20-22]. In addition, tumour VM is correlated with metastatic tumours and closely associated with poor prognosis in cancer patients[23-25].

Tumour vascular characteristics

Aggressive growth of the tumour cell population and overexpression of proangiogenic factors lead to vascular network disorder. The typical characteristics of tumour vascular system are abnormal structural dynamics, immature, tortuous and hyperpermeable vasculature[9,26,27]. The abnormal characteristics lead to aberrant microenvironmental conditions that obstruct traditional anticancer treatment strategies[28]. Microregional hypoxia can lead to resistance to

radiotherapy and chemotherapy. Despite these phenomena, the unique characteristics of the tumour vascular system also present an opportunity for selective therapeutic interventions compared to the normal tissue vasculature[29].

Current status of antiangiogenic agents in OC

Bevacizumab is a humanized anti-VEGF monoclonal antibody that was the first to be widely studied and is currently the most widely applied antiangiogenic drug in a variety of tumours, including epithelial OC[30]. In 2014, bevacizumab was approved by the US Food and Drug Administration for the treatment of platinum-resistant epithelial OC. There have been several randomized phase III clinical trials that evaluated bevacizumab combined with first-line chemotherapy for recurrent OC. The results of these trials showed that the PFS of OC patients improved, but the benefit in patient OS was modest (Table I)[31]. ICON-7 and GOG-

218 were the first two front-line phase III trials that tested bevacizumab in combination with chemotherapy (carboplatin and paclitaxel)[32,33]. ICON-7 enrolled 1528 OC patients, 70 per cent of whom were stage IIIc or IV, the median PFS of bevacizumab arm was significantly improved (19.8 months *vs.* 17.4 months). GOG-218 was a placebo-controlled three-arm study (paclitaxel + carboplatin + placebo; placebo maintenance versus paclitaxel + carboplatin + bevacizumab; placebo maintenance versus paclitaxel + carboplatin + bevacizumab; bevacizumab maintenance), the median PFS of bevacizumab throughout the arm was significantly improved (3.8 months). Three randomized phase III trials (GOG-213, OCEANS and AURELIA)

evaluated bevacizumab in recurrent cases of OC. GOG-213 enrolled 674 platinum sensitive relapse patients. The addition of bevacizumab significantly extended PFS (13.8 *vs* 10.4 months), but there was no significant improvement in OS (*P*=0.56, HR: 0.83)[36]. OCEANS trial evaluated the benefits of adding bevacizumab to gemcitabine and carboplatin in platinum sensitive patients. The results showed that PFS improved for four months, but no improvement was observed in OS[35]. The AURELIA trial showed that combined bevacizumab with cytotoxic regimens extended the PFS of platinum resistant patients (6.7 *vs* 3.4 months)[34]. Other antiangiogenic agents that target the VEGF/VEGF receptor (VEGFR) signalling pathway are multitarget tyrosine kinase inhibitors (TKIs) and corresponding phase III clinical trials have been carried out, including nintedanib (OVAR-12)[37], cediranib (ICON6)[38] and pazopanib (OVAR-16)[39]. These TKIs have multiple targets that are different from those of bevacizumab (Table II); although their mechanism of action is more attractive than that of bevacizumab, TKIs do not show significant advantages in terms of patient prognosis compared with drugs that target VEGF alone[41], such as bevacizumab (Table III). In addition, because of the wide range of targets, there is the possibility of severe toxic side effects. OC has been treated with a variety of antiangiogenic drugs so far, but the results so far

Table II. Targets of tyrosine kinase inhibitors that were studied in phase III clinical trials of epithelial ovarian cancer

Agent	Route of administration	Targets
Nintedanib[37]	Oral	VEGFR, FGFR, and PDGFR
Pazopanib[39]	Oral	VEGFR, PDGFR, FGFR, c-Kit, and c-Fms
Cediranib[40]	Oral	VEGFR

VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor

Table III. Phase III studies of anti-angiogenic agents that target tyrosine kinase receptors in ovarian cancer

Study	Agent	n	Setting	Treatment arm	Clinical outcomes [media PFS, months, HR (95%CI)]
OVAR-12[37]	Nintedanib	1366	Front-line and maintenance	Paclitaxel+carboplatin+placebo; placebo maintenance versus paclitaxel+carboplatin+nintedanib; nintedanib maintenance	PFS: 16.6 *vs.* 17.3 (HR=0.84, *P*=0.024)
OVAR-16[39]	Pazopanib	940	Maintenance	Placebo versus pazopanib	PFS: 12.3 *vs.* 17.9 (HR=0.77, *P*=0.0021)
ICON6[38]	Cediranib	456	Recurrent, platinum-sensitive	Chemotherapy (paclitaxel or gemcitabine combination) or single agent carboplatin+placebo; placebo maintenance versus chemotherapy+cediranib; placebo maintenance versus chemotherapy+cediranib; cediranib maintenance	PFS: 8.7 *vs.* 10.1 *vs.* 11.1 HR=0.67 (0.53-0.87)[#] HR=0.57 (0.44-0.74)[†]

Source: Adapted with permission from Ref 31. [#]Chemotherapy (paclitaxel or gemcitabine combination) or single agent carboplatin+placebo; placebo maintenance versus chemotherapy+cediranib; placebo maintenance; [†]Chemotherapy (paclitaxel or gemcitabine combination) or single-agent carboplatin+placebo; placebo maintenance versus chemotherapy+cediranib; cediranib maintenance; PFS, progression-free survival; OS, overall survival; HR, hazard ratios; CI, confidence interval

have not been promising. Although the PFS has been observed to be prolonged in some clinical trials, the OS has not improved significantly.

Mechanisms of resistance and biomarkers

Because of the high cost and potential side effects of these drugs, only a small percentage of patients can benefit; therefore, a better understanding of bevacizumab resistance mechanisms and the identification of predictive biomarkers are essential.

Endogenous or acquired resistance is considered to be the main cause of failure in antiangiogenic therapy[42]. Based on some preclinical studies, several drug resistance mechanisms against vascular therapy have been proposed. First, antiangiogenic therapy aggravates tumour hypoxia, which leads to elevated hypoxia inducible factor 1A (HIF1A) levels, the stimulation of angiogenesis-related factors, such as fibroblast growth factors (FGFs), angiotensins (ANGs) and interleukin-8, and a high risk of tumour invasion and metastasis[43-45]. Second, when VEGF activity is neutralized, blood perfusion in the tumour tissue is significantly reduced, thereby impairing the killing effect of chemotherapy and radiotherapy on the tumour. Third, anti-VEGF therapy blocks the VEGF/VEGFR-dependent angiogenic pathways but results in the upregulation of VEGF-independent angiogenesis mechanisms, such as those associated with angiopoietin 1 (Ang1), Dll4/Notch and microRNAs (miRNA)[46]. Fourth, immune responses can lead to the recruitment of pro-angiogenic monocytes from the bone marrow, induction of hypoxia, or high pericyte coverage of the tumour vascular system[47]. According to data from 2011 from the Cancer Genome Atlas, HGSOC is divided into four subtypes, and among them proliferative and mesenchymal subtypes are associated with poorer survival, but derive a comparably greater benefit from treatment of bevacizumab than the other two subtypes. In contrast, bevacizumab conferred modest improvements in the PFS of patients with the immunoreactive subtype or differentiated subtype[48]. The above data indicate that patients should be classified when receiving vascular-targeted drugs, such as bevacizumab, to ensure the efficacy of the targeted drugs, reduce side effects, as well as medical costs. Fifth, the heterogeneity of tumour cells allows some tumour cell subsets to survive under hypoxic conditions, thereby increasing the risk of invasion and metastasis. The heterogeneity of the tumour vasculature itself in tumour tissue represents a difference in demand for

VEGF[49,50]. This may be the most critical mechanism to explain endogenous antiangiogenic resistance. Four angiogenic patterns are present in OC tissues, as mentioned above, three of which are nonvascular endothelium dependent. Differences between individuals, different proportions of vascular subtypes in different tumour tissues, and changes in the ratio between VEGF-dependent and VEGF-independent vascular subtypes during antiangiogenic therapy lead to resistance to antiangiogenic therapy. Several other candidate markers, such as plasma protein levels, circulating endothelial cells, and free DNA, have also been proposed, but have not been verified[51].

Prospects for antivascular therapeutic targets

The application of vascular-targeted therapy in OC is not successful. In addition, in breast cancer and liver cancer, studies have shown that antivascular therapy increases the risk of tumour invasion and metastasis[52-54], but the reason is so far unclear. One possible mechanism is the lack of oxygen. Hypoxia is a typical characteristic of most solid tumours and is related to the overexpression of hypoxia response pathway molecules. Overexpression of HIF1 protein leads to enhanced tumour angiogenesis, proliferation, invasion, metastasis and apoptosis resistance. Therefore, the HIF1 protein is a promising target for improving the sensitivity to chemoradiotherapy in cancer patients and improving the survival rate. However, no HIF1 protein inhibitor has been applied in clinical research[55].

The phase III clinical trials of OC that combined inhibitors targeting VEGF/VEGFR angiogenic pathways with various chemotherapy drugs found that PFS was significantly improved in patients, but the difference in OS was not significant[34]. In response to this phenomenon, some scholars have proposed the concept of tumour vascular normalization which indicates that chemotherapy and radiotherapy combined with VEGF/VEGFA angiogenesis signalling pathway inhibitors can promote tumour vascular maturation and improve clinical symptoms in a short span of time[56]. The secretion of large amounts of VEGF by tumour cells results in the formation of immature blood vessels that lack pericyte coverage. A certain dose of VEGF/VEGFR inhibitor can restore the tumour angiogenesis signal to a certain extent. By strengthening the tight link between cells, actively recruiting pericytes, reducing tumour vascular permeability, and increasing blood flow perfusion of tumour tissue, the sensitivity to radiotherapy or chemotherapy can be increased.

However, not all patients can benefit from this finding. Studies have shown that patients with high microvessel density (MVD) expression have high sensitivity to VEGF inhibitors, so the basal expression of MVD can be used as a marker to determine whether bevacizumab will be effective[57,58]. In addition, antagonizing VEGFR2 activates the Ang- 1/Tie2 signalling pathway, thereby recruiting pericytes[59]. At the same time, inhibition of VEGF can upregulate PDGFRB signalling and promote the recruitment and maturation of pericytes[60]. Another challenge in vascular normalization with VEGF inhibitors is achieving what is refered to as a 'window of opportunity', which is the time frame and dose of VEGF inhibitor that are required to observe normalization of tumour blood vessels. The dose time frame and the amount of VEGF inhibitors required to attain normalization were relatively narrow, depending on the type of tumour used, dose planning and VEGF signalling inhibitors. The effect is usually transient (7-10 days), but could last 1-4 months, depending on the drug used and the type of tumour[61-63]. To improve the sensitivity of chemotherapy, radiotherapy and immunotherapy, it is necessary to maintain normalization of the tumour blood vessels for a long time; therefore, it is essential to develop an effective and long-term strategy for stabilizing the tumour blood vessels[63]. Three major functional imaging techniques can be used to evaluate patients undergoing antiangiogenic therapy: dynamic contrast-enhanced (DCE)-US, DCE computed tomography (CT), and DCE magnetic resonance imaging (MRI) which can determine the function of the tumour blood vessels after antiangiogenic therapy and help determine the ideal treatment dosage for normalization[64-67]. In addition, tracers can also be used to monitor vascular normalization after VEGF inhibitor treatment. A specific radiotracer ^{99m}TcRGD binds to integrin avb3, which is expressed during active angiogenesis and has been shown to help in monitoring vascular normalization after bevacizumab treatment and helps to identify the solution required for an ideal efficacy rate[68].

The Dll4/Notch signalling pathway is crucial to the development of embryonic blood vessels, and studies have shown that it is also closely related to tumour angiogenesis[69]. Changes in the Notch signalling pathway are common in HGSOC and are associated with low OS[70]. Dll4 is an endothelial-specific ligand that is highly expressed in tumour blood vessels, and 72 per cent of OC patients exhibit Dll4 overexpression,

which is an independent risk factor for poor prognosis. The expression of Dll4 is low in patients who are sensitive to VEGF inhibitors, and knockdown of the *Dll4* gene in ovarian tumour cells and tumour-associated endothelial cells leads to low tumour growth and angiogenic capacity[71]. Therefore, Dll4/Notch may be a potential target for vascular-targeted therapy in OC.

Numerous studies have shown that inflammation promotes tumour progression. Tumour-associated macrophages (TAMs) are the main inflammatory components of tumour stroma, which are associated with tumour development and anti-vascular resistance[72-77]. Cytokines in the tumour microenvironment polarize TAMs toward an M2 phenotype, which is characterized by high expression of IL-10, TGFβ, VEGF, MMPs and other cytokines that inhibit adaptive immunity, stimulate metastasis and angiogenesis[78-80]. In addition, TAMs accumulated in tumour hypoxia areas, and hypoxia induced by antiangiogenic therapy was associated with high TAM infiltration[63]. *In vivo* experiments have shown that compared to control groups, groups with macrophages depleted from the abdominal cavity have reduced ascites production and inhibited tumour growth and angiogenesis[81]. Compared with sorafenib alone, ZA combined with sorafenib can obviously inhibit tumour progression, metastasis and tumour angiogenesis in a mouse model of liver cancer by targeting macrophages[82]. Neferine inhibits tumour growth by inhibiting the differentiation of M2 macrophages and inducing autophagy to inhibit angiogenesis in high-grade serous OC[83]. Hence, TAMs are a promising target for the treatment of OC.

MiRNA is a noncoding small RNA, and an increasing number of studies have shown that miRNA plays an important role in tumour angiogenesis[84-86]. A single miRNA can target hundreds of mRNA transcripts for translational inhibition, mRNA degradation, or induction of mRNA instability to regulate target gene expression[87]. This feature allows miRNAs to simultaneously target multiple angiogenesis-related pathways. Wu *et al*[88] showed that miR-192 can target multiple angiogenesis-related genes and mediate potent antiangiogenic and antitumour effects in multiple orthotopic mouse models of ovarian and renal cancer and that the antiangiogenic and antitumour effects of miR-192 were stronger than those of VEGF antibodies. Through large-scale patient data, lower levels of miR-192 in tumours were shown to be associated with high angiogenesis and low overall survival in patients with
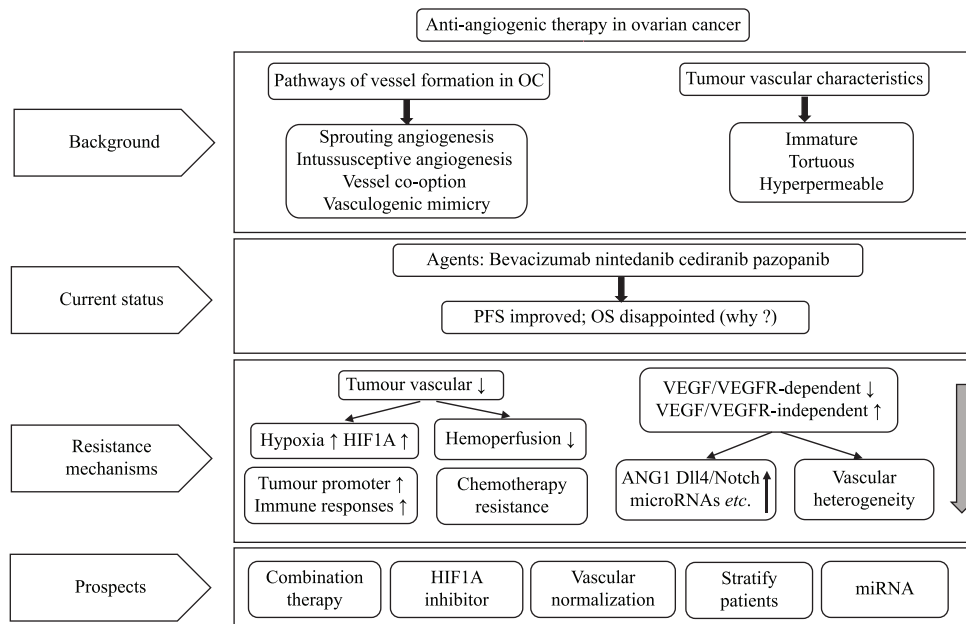
Fig. 2. An outline diagram summarizes the entire review narrative on angiogenesis.

HGSOC or renal clear cell carcinoma. Chen *et al*[5] proved that miR-204-5p could promote angiogenesis in ovarian tumours through THBS1. Therefore, miRNA is a potential target for the treatment of OC.

Status of targeted therapy for OC

The combination of paclitaxel and carboplatin every three weeks with or without bevacizumab remains the first-line standard treatment for advanced OC patients[89]. At present, it is believed that the survival rate of OC patients cannot be improved by either dose-dense chemotherapy or adding a third chemotherapy drug or intraperitoneal (IP) therapy administration[90]. GOG-172 was a phase III trial with OS is reported to improve by 15.9 months (65.6 *vs* 49.7 months) in the IP arm compared with the intravenous (IV) arm. Due to increased catheter-related complications and toxicity, only 42 per cent patients completed the six cycles of the assigned therapy. GOG-252 is a phase III trial to further evaluate the role of IP compare with IV chemotherapy, the results failed to show a survival benefit from IP chemotherapy[91] due to which it is not universally accepted[92,93]. In addition, hyperthermic intraperitoneal chemotherapy (HIPEC) or neoadjuvant chemotherapy is not better than standard chemotherapy. Although HIPEC is widely used, up to now, there was no randomized and convincing evidence for HIPEC versus surgery without HIPEC for OC[94]. Based on studies on the pathogenesis of OC, several target agents have been used in the treatment of OC. Targeted agents

are less toxic than chemotherapy drugs and can be combined[95,96]. Currently, several combination trials are ongoing, including trials of antiangiogeneic drugs with poly-(ADP)-ribose polymerase inhibitors (PARPi) [PAOLA-1 or ENGOT-ov25 trial (NCT0247764)], immune checkpoint inhibitors plus antiangiogenic agents [IMaGYN050 (NCT03038100)] and the combination of antiangiogenic agents, PARPi and checkpoint inhibitors (ENGOT-ov46 trial)[97,98]. These ongoing OC trials certainly show great promise, and we eagerly await the results of these studies.

Conclusion

The main contents of this review are summarized in Fig. 2. All in all, antiangiogenic strategies are key for OC therapeutic management, with less toxicity than conventional chemotherapy methods, and can be used as a potential maintenance therapy to reduce or delay recurrence. However, to date, there is a lack of effective measures to classify OC patients and effectively determine which patients may benefit from VEGF/VEGFR inhibitors, which makes these interventions costly, minimizes efficacy and increases side effects. To improve this shortcoming, the following aspects should be considered in future studies of OC angiogenesis. First, there are multiple mechanisms involved in OC angiogenesis. To effectively inhibit angiogenesis, multiple angiogenic pathways need to be blocked simultaneously, and miRNAs may be an ideal therapeutic approach in this context. Second, there is

high heterogeneity of tumour blood vessels in tumour tissues, and future research directions should include identifying predictable biomarkers to identify which patients are responders. Third, vascular normalization is a new therapeutic strategy, which has great clinical potential in improving the local immunosuppressive microenvironment, enhancing drug delivery, and improving the hypoxia status of tumours. Therefore, the challenges in future studies will involve determining the optimal duration and the scheduling of vascular normalization agents and how to combine different agents effectively without significant toxicity. Ultimately, to achieve this goal, a close cooperation between basic researchers and clinicians is essential.

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