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Mechanism of action of Salvianolic acid A in tumor angiogenesis inhibition and vascular normalization

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Abstract. Tumor growth is usually accompanied by the formation of abnormal vascular networks, and these abnormal vessels promote malignant biological behavior of tumors. Salvianolic acid A (SAA) has shown promising effects in inhibiting tumor angiogenesis and promoting vascular normalization. This paper reviews the mechanism by which Salvianolic acid A inhibits angiogenesis by ameliorating hypoxia in the tumor microenvironment and blocking the secretion of glucose-regulated protein 78 (GRP78). In addition, Salvianolic acid A normalizes tumor vasculature by stabilizing vascular endothelial cell junctions and limiting glycolysis. The aim of this paper is to provide a theoretical basis for the application of Salvianolic acid A in tumor therapy and a reference for further research.

Keywords: Salvianolic acid A, Tumor angiogenesis, Normalization of tumor vasculature.

1. Introduction

The tumor microenvironment (TME) refers to the complex surrounding environment of tumor cells, which includes non-malignant host cells, for example, endothelial cells, fibroblasts, and immunological cells, an extracellular matrix (ECM) that supports cell proliferation and communication, adjacent blood vessels, and soluble factors like chemokines, growth factors, and cytokines. These components of the TME are crucial in promoting processes such as cell migration, angiogenesis, immune suppression, and drug resistance, all of which contribute to tumor progression and therapeutic outcomes [1]. The composition and influence of the TME vary significantly across different cancer types [2].

During tumor progression, the TME undergoes significant changes, with one key feature being the release of angiopoietin (Ang) and vascular endothelial growth factor (VEGF), two pro-angiogenic factors. These factors stimulate surrounding blood vessels, induce abnormal proliferation, and ultimately lead to the formation of tumor-specific abnormal vascular networks. Compared to normal blood vessels, tumor vessels are highly unstable, prone to hemorrhage, and promote malignant behaviors such as recurrence, metastasis, immunosuppression, and drug resistance. Thus, repairing and normalizing these abnormal tumor vessels can improve the efficacy of anti-tumor therapies. Preventing tumor angiogenesis and prolonging the "time window" of vascular normalization has become a viable therapeutic approach for the treatment of cancer [3].

Salvianolic acid A (SAA), a key bioactive compound derived from the root of Salvia miltiorrhiza, is known for its ability to counteract oxidative stress by scavenging free radicals, inhibiting apoptosis via modulation of signaling pathways like MAPK, PI3K/Akt, and mTOR, and regulating inflammation through the suppression of pro-inflammatory mediators such as IL-6 and TNF-α [4]. Recent studies

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indicate that SAA also plays a significant role in regulating tumor blood vessels. By improving hypoxia within the tumor microenvironment and inhibiting the secretion of GRP78 in tumor exosomes, SAA can prevent abnormal tumor angiogenesis. Moreover, SAA helps normalize tumor vessels by strengthening endothelial cell connections and restricting endothelial cell glycolysis. Thus, SAA holds potential as an effective therapeutic agent for preventing abnormal angiogenesis and promoting tumor vascular normalization. This review aims to summarize current research on SAA's mechanisms in regulating tumor vasculature, providing insights into its potential clinical applications.

2. Mechanism of Action of SAA in Regulating Tumor Angiogenesis

2.1. SAA Inhibits Angiogenesis by Improving Hypoxia in the Tumor Microenvironment

During the rapid proliferation of tumor cells, their surrounding nutrients are consumed in large quantities, accompanied by high oxygen consumption, insufficient nutrient supply, and accumulation of cellular metabolites, which ultimately leads to hypoxia in the tumor microenvironment. HIF-1α, HIF-2α, and HIF-1β combine to form the transcription factor known as hypoxia-inducible factor (HIF). Under normal oxygen conditions, HIFα is hydroxylated and then degraded, but in a hypoxic environment, this degradation process is inhibited, and HIFα binds to HIFβ to form a dimer that enters the nucleus and activates downstream hypoxia-responsive elements (HREs), thus promoting the expression of genes related to angiogenesis.HIF-1α plays a key regulatory role in this process, and induces the expression of these genes through its binding to the promoters of vascular endothelial growth factor (VEGF) and its receptors (VEGFRs), inducing the expression of these genes and thus promoting tumor angiogenesis. In addition, HIF-1a reduces the expression of anti-angiogenic molecules, further enhancing angiogenesis. Therefore, the tumor hypoxic microenvironment plays a crucial role in angiogenesis [5].

After SAA treatment, the expression levels of the classical hypoxia-related indicators HIF-1α and carbonic anhydrase-9 (CA-9) were significantly reduced; HIF-1α, as a key transcription factor activated under hypoxic conditions, is involved in the regulation of multiple genes related to hypoxia adaptation; while CA-9 is closely related to the acidic and hypoxic environment of tumors. The reduction of these indicators suggests that SAA can effectively reduce the degree of hypoxia in the tumor microenvironment and thus inhibit tumor angiogenesis [6]. In addition, SAA accelerates HIF-1α degradation by regulating the Akt/GSK-3 axis. Glycogen synthase kinase-3β (GSK-3β) is an important mechanism to reduce the stability of HIF-1α, and SAA reduces the phosphorylation of Akt at the classical Ser473 residue, thereby inactivating Akt, which ultimately leads to the enhanced activity of GSK-3β and promotes the degradation of HIF-1α and prevents angiogenesis [7]. This mechanism has also been shown to enhance the effectiveness of chemotherapeutic agents, as seen in a breast cancer model where SAA combined with paclitaxel significantly increased tumor suppression [8].

2.2. SAA Inhibits Tumor-Associated Angiogenesis by Blocking GRP78 Secretion

Glucose Regulatory Protein 78 (GRP78, also known as BiP) is mainly located in the endoplasmic reticulum (ER) and serves as a chaperone protein to help proteins fold and assemble correctly. In tumor cells, GRP78 is also selectively secreted into the extracellular environment via membrane vesicles to promote tumor angiogenesis. Overexpression of GRP78 promotes endothelial cell proliferation, inhibits apoptosis, and enhances their migratory ability, thereby contributing to the establishment of tumor vascular networks. GRP78 also promotes the expression of angiogenic factors by interacting with transcription factors such as ID2 [9]. In addition, GRP78 is involved in a range of signaling pathways, such as the PI3K/AKT signaling pathway, which play an important role in tumor angiogenesis.

The lysine 633 position in the substrate binding domain (SBD) of GRP78 was found to be a key regulatory site for its secretion. Through virtual screening and molecular docking technology, three-dimensional interaction models of several small molecule drug structures and GRP78 protein structures were established, and it was found that many small molecules of traditional Chinese medicine and GRP78 protein structures had strong interaction and multiple binding modes. Among them, SAA, an inhibitor with strong affinity for GRP78, could bind to K633 in the GRP78 substrate-binding domain

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(SBD) and interact with GRP78, and it was also found that SAA could significantly reduce the amount of GRP78 secreted in tumor cells. Further studies showed that the formation of the SAA-GRP78 complex induced the sorting of GRP78 in the cytosol into the lysosomal degradation pathway rather than secretion into the extracellular compartment via exosomes, thereby interfering with GRP78 secretion and inhibiting tumor angiogenesis [10].

3. Role of SAA in Tumor Vascular Normalization

3.1. SAA Inhibits Angiogenesis by Improving Hypoxia in the Tumor Microenvironment

Claudin-5 is an important component of tight junctions, mainly in vascular endothelial cells, and its expression promotes the formation of tight junctions, thereby reducing vascular permeability [11]. β-Catenin is a multifunctional cytoskeletal protein that not only plays an important role in the maintenance of cell morphology and polarity, but also in the formation and regulation of intercellular junctions. In endothelial cells, β-Catenin interacts with tight junction proteins, such as Claudin-5, in the structural and functional maintenance of tight junctions, and also influences the dynamics of tight junctions by regulating the activation and inhibition of related signaling pathways. Under normal conditions, this interaction helps to reduce unnecessary material exchange and prevent vascular leakage, thus maintaining normal vascular structure and function [12]. Studies have shown that PKM2 can act as a coactivator of β-Catenin and participate in the transcriptional regulation of various genes (including CLDN1 and CLDN5) to enhance endothelial cell connectivity, and SAA can promote the nuclear translocation of PKM2 to β-Catenin and reduce the phosphorylation level of β-Catenin, enhance its binding to TCF4, and ultimately enhance the tight junction of endothelial cells. endothelial cell tight junctions and prevented vascular leakage. In addition, SAA also up-regulated the mRNA expression levels of CTNNB (the gene name of β-Catenin) and its downstream target genes, which further strengthened its regulatory effect. It can be concluded that SAA treatment enhances the binding of β-Catenin to PKM2 and the interaction between β-Catenin and TCF4, which in turn enhances the tight junctions of endothelial cells, reduces the unnecessary exchange of substances, prevents vascular leakage, and achieves the goal of promoting vascular normalization [6].

3.2. SAA Limits Endothelial Cell Glycolysis and Stabilizes Vascular Structure by Binding to PKM2

Glycolysis is a way for cells to obtain energy, especially under hypoxic conditions, and cells rely on glycolysis to generate Adenosine triphosphate. Tumor angiogenesis requires a large amount of energy to support the proliferation and migration of vascular endothelial cells and the formation of vascular structures. Glycolysis provides the necessary energy support for this process, enabling endothelial cells to respond rapidly to the demands of tumor growth and form new vascular networks[13]. PKM2 is a key enzyme in the glycolytic pathway and exists in both dimeric and tetrameric forms, with PKM2 in the tetrameric state showing a stronger affinity for its substrate, phosphoenolpyruvate (PEP), and further displaying higher PK activity, thus catalyzing the production of pyruvate from PEP. PKM2 can also be maintained in the dimeric state with low PK activity, and the dimeric PKM2 has the ability to enter the nucleus, thereby promoting the transcription of multiple genes. After SAA treatment, the expression level of PKM2 tetramers was reduced, while the relative level of dimers was up-regulated. This conformational change was able to affect the enzymatic activity of PKM2, resulting in a decrease in overall PK activity, which in turn reduced the rate of the glycolytic pathway. Moreover, there is a direct interaction between SAA and PKM2, and molecular docking analysis was able to predict the binding mode of SAA to the catalytic structural domain of PKM2, and found that SAA interacts with several residues within PKM2, including Arg489, Gly520 and Lys433. Among them, the Lys433 residue is believed to be the key site mediating the binding of SAA to PKM2. Therefore, SAA can stabilize the vascular structure by directly interacting with the Lys433 residue of PKM2, inhibiting its enzymatic activity and changing its conformation, which in turn affects the rate of the glycolytic pathway [6].

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4. Discussion

The study of the mechanism of action of SAA in preventing tumor angiogenesis and promoting tumor vascular normalization provides new perspectives for understanding tumor vascular abnormalities and tumor progression, as well as new strategies for tumor therapy. Researchers can gain a deeper understanding of the complex processes that drive tumor growth and metastasis and design more targeted and effective therapeutic approaches that specifically target potential abnormalities in the tumor vascular system. It is expected to improve patient outcomes and advance the field of cancer research. SAA effectively slowed down tumor growth and proliferation by inhibiting tumor angiogenesis. Hypoxia is a common phenomenon within the tumor microenvironment, usually characterized by low oxygen levels and nutrient deprivation, which can create an environment conducive to tumor growth and resistance to treatment. And SAA was able to accelerate the degradation of HIF-1α by regulating the Akt/GSK-3 axis, preventing angiogenesis; at the same time, SAA was also able to bind to GRP78, forming a SAA-GRP78 complex, which prompted GRP78 in the cytosol to be degraded by the lysosome to be unable to be secreted, thus inhibiting tumor angiogenesis.

Moreover, SAA showed remarkable effects in promoting tumor vascular normalization. SAA was able to promote the enhancement of vascular endothelial cell connectivity, which is essential for maintaining vascular integrity and function, by enhancing the binding of β-Catenin to PKM2 and the interaction of β-Catenin with TCF4. This action enhances the stability of the tumor vascular system, reduces leakage, and improves blood flow. SAA also stabilizes vascular structure by limiting glycolysis through inhibition of PKM2 activity and altering its conformation. SAA exerts anti-tumor effects by regulating key signaling axes such as Akt/GSK-3, GRP78, and β-Catenin/Claudin-5. These signaling pathways play crucial roles in cell proliferation, survival, and migration, and their dysregulation is frequently observed in cancer. By targeting these pathways, SAA can significantly improve the therapeutic efficacy of combination chemotherapeutic agents, making it a promising adjuvant cancer therapy.

Notably, the anti-tumor angiogenic and vascular normalization-promoting effects of SAA are not isolated phenomena but rather form a comprehensive strategy in the fight against cancer. Several experiments have robustly confirmed that when SAA is used in combination with chemotherapeutic drugs such as paclitaxel, it can significantly enhance the efficacy of these drugs. This synergistic effect not only augments the direct cytotoxic action of paclitaxel but also reduces the risk of tumor recurrence and metastasis. This beneficial interaction may be attributed to SAA's ability to improve the tumor microenvironment by normalizing blood vessel structure and function, thereby enhancing the permeability and distribution of chemotherapeutic agents within the tumor tissue.

However, despite the promising results demonstrated by SAA in anti-tumor angiogenesis and promotion of vascular normalization, its intricate mechanism of action still requires further in-depth exploration. For instance, we need to delve deeper into how SAA regulates endothelial cell metabolism and signal transduction pathways. Understanding these regulatory mechanisms will provide insights into how SAA affects the structure and function of tumor vessels, ultimately leading to improved therapeutic outcomes. In addition, to fully harness the potential of SAA in cancer therapy, it is imperative to conduct more extensive and rigorous clinical trials. These trials should not only validate the safety and efficacy of SAA but also explore optimal dosing strategies and potential synergistic combinations with other therapeutic agents. By gathering comprehensive data from clinical trials, we can provide robust evidence to support the wide application of SAA in tumor therapy, ultimately contributing to better patient outcomes and improved quality of life for cancer patients.

In summary, SAA represents a promising antitumor agent, offering novel therapeutic avenues through its dual mechanisms of inhibiting tumor angiogenesis and promoting vascular normalization. Further investigations are warranted to deepen our understanding of SAA’s antitumor effects and assess its clinical viability as a therapeutic intervention in oncology.

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5. Conclusion

SAA is a natural compound from Salvia miltiorrhiza, that exerts a multifaceted regulatory effect on tumor angiogenesis and vascular normalization. By improving the hypoxic state of the tumor microenvironment, SAA attenuates conditions conducive to aggressive tumor growth and treatment resistance, while preventing the secretion of this key factor mediating tumor vascular regeneration by binding to GRP78, which in turn inhibits tumor angiogenesis. In addition to inhibiting angiogenesis, SAA also enhances endothelial cell connectivity. It reduces the destruction of vascular endothelial cells and prevents abnormal permeability and leakage of blood vessels. In addition, SAA restricts glycolysis, the metabolic process by which cancer cells convert glucose into energy under hypoxic conditions. This disruption of the tumor's metabolic pathways further diminishes its ability to sustain growth and resist treatment. SAA inhibits the tumor vascular system and enhances sensitivity to combination chemotherapy by disrupting signals that promote angiogenesis and aberrant vascular growth through regulation of key signaling pathways such as the Akt/GSK-3 axis, GRP78, and the β-Catenin/Claudin-5 axis. The role of SAA in tumor therapy provides an important basis for further research, and further study of SAA's mechanism of action and optimization of dosing strategies will be key to maximizing its potential for clinical application in the future. Understanding how SAA interacts with different tumor types and genetic backgrounds will help to achieve more personalized and effective therapeutic approaches, ultimately providing better patient outcomes for cancer treatment.

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