Overview of serpin B9 and its roles in cancer (Review)

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Abstract. Serine proteinase inhibitor B9 (serpin B9) is a member of the serine protease inhibitor superfamily, which is widely found in animals, plants and microorganisms. Serpin B9 has been reported to protect cells from the immune-killing effect of granzyme B (GrB) released by lymphocytes. In recent years, an increasing number of studies have indicated that serpin B9 is involved in tumour apoptosis, immune evasion, tumorigenesis, progression, metastasis, drug resistance and even in maintaining the stemness of cancer stem cells (CSCs). Moreover, according to clinical studies, serpin B9 has been demonstrated to be significantly associated with the development of precancerous lesions, a poor prognosis and ineffective therapies, suggesting that serpin B9 may be a potential target for cancer treatment and an indicator of cancer diagnosis; thus, it has begun to attract increased attention from scholars. The present review concisely described the structure and biological functions of the serpin superfamily and serpin B9. In addition, related research on serpins in cancer is discussed in order to provide a comprehensive understanding of the role of serpin B9 in cancer, as well as its clinical significance for cancer diagnosis and prognosis.

Contents

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1. Introduction

According to clinical statistics, the number of new cases and cancer-related deaths each year remain high, bringing a heavy burden to health care and economy systems worldwide (1,2). Tumorigenesis and development of cancer are influenced by various factors, such as genes, the environment and current treatment strategies; thus, it is extremely challenging to identify new targets and antitumor drugs (3). It has been revealed that the immune evasion of cancer, a process through which cancer cells evade the immune surveillance system of the body, is involved in tumorigenesis and cancer progression (4). Therefore, immunotherapies based on the immune evasion of cancer have been developed; as typical examples, treatments targeting the anti-program death-1 and anti-program death-ligand 1 pathways have been widely utilized in various types of cancer, such as lung cancer, lymphoma, melanoma and liver cancer (5).

A previous study demonstrated that the knockdown of serine proteinase inhibitor B9 (serpin B9) led to the granzyme B (GrB)-dependent death of lung cancer cells, and serpin B9 inhibitors not only increased the apoptosis of lung cancer cells, but also inhibited tumour growth and prolonged the survival time of mice with lung cancer (6). Serpin B9, as a promising target molecule of antitumour treatment, has been revealed to protect cancer cells by enhancing immune evasion; it has thus attracted increased attention from scholars. Therefore, the aim of the present review was to summarise the current knowledge on the role of serpin B9 in cancer and provide information on its discovery, structure, functions and association with cancer.

2. Serpin superfamily

Serpin is a superfamily of protease inhibitors with a common source and highly homologous structural sequence, yet with highly differentiated functions, which was first discovered in human plasma and obtained through separation and purification (7-10). Serpin is widely found in the natural world, including in bacteria, fungi, plants and animals (11). Serpin, as a single-chain protein, is usually composed of 350-400 amino acids, with a molecular weight between 40-100 kDa (8). Thus far, this superfamily has been identified in >1,000 species, and is divided into 16 subgroups (A-P) in terms of different

origin (11). All serpins share a conserved tertiary structure made of three β-sheets, eight or nine α-helices and a reactive centre loop (RCL), which contains 17 amino acids and is located between the A and C β-sheets (11). The RCL is the main action site located in the protease recognition domain close to the C-terminus of serpin (12) and determines the specificity of serpin inhibitory functions (13,14). Previous research has demonstrated that there are two types of serpin inhibitory mechanisms, termed the inhibitory pathway and the substrate pathway (Fig. 1). At first, serpin and protease interact and form a compound named the reversible Michaelis complex. Subsequently, according to the stoichiometry of inhibition (SI), which is divided by 1, the complex undergoes different pathways. When the SI is close to 1, the irreversible inhibitory complex is formed through inhibitory pathway; when the SI is >1, cleaved and inactivated serpin and intact protease remain through the substrate pathway (15). However, the pathway of serpin B3 and serpin B4, which also leads to a decreased function of protease, has not yet been elucidated (10,11).

An increasing number of studies have demonstrated that serpin inhibits proteases associated with inflammation, the complement system, blood coagulation, immunity and other processes, in various pathophysiological processes and diseases (16-18). An example of this is the role of serpin members in cancer. Specific serpins are associated with the development or inhibition of tumours, thus rendering them valuable as diagnostic or therapeutic targets in cancer. Serpin B2, also known as plasminogen activator inhibitor type 2 (PAI-2), is a regulator of thrombolysis in the body (19). The inhibition of PAI-2 has been revealed to be involved in decreasing the migration, proliferation and metastasis of cancer cells via the modulation of the function of urokinase-type plasminogen activator receptor, which suggests that PAI-2 may become a biomarker for cancer diagnosis or a target for cancer treatment (20). Serpin B3, initially known as squamous cell carcinoma antigen-1, is also a member of the serpin superfamily (21). It has been found that serpin B3 is involved in chronic liver injury, which is considered as having a high risk to develop into liver cancer, including the inhibition of liver cell apoptosis, the induction of epithelial-to-mesenchymal transition, and increasing liver cell proliferation and invasiveness (22). Serpin F1 [pigment epithelium-derived factor (PEDF)] has been revealed to be upregulated in certain tumour cells, such as breast cancer cells (23,24). Further studies have indicated that, compared with the normal expression of PEDF in tumours, the decreased intra-tumoural expression of PEDF is related to a higher microvessel density, a more metastatic phenotype and a poorer clinical outcome of breast cancer; thus, PEDF may be a valuable prognostic biomarker for cancer (23,24). An increasing number of studies have thus demonstrated that serpin plays a significant role in cancer. Therefore, it may be a promising biomarker for early diagnosis, an important predictor of prognosis and a potential therapeutic target. In the serpin superfamily, serpin B9 is receiving increased attention from scholars.

3. Serpin B9 structure and functions

The clade B serpins have 13 members of the B subgroup in humans, namely serpin B1-serpin B13, which lack signal peptides and are primarily located intracellularly (25,26). Serpin B9 is a single-chain protein consisting of 376 amino acids with a molecular weight of 42 kDa, which was first screened from the human placental cDNA library and whose genes are located at chromosome 6p25, containing seven exons and six introns (27-29). Serpin B9 has a highly conserved tertiary structure, which includes nine α-helices, three β-sheets and an RCL at the carboxyl end (15) (Fig. 2). The RCL of serpin B9 has a specific PI-PI' site which consists of a highly conserved cysteine pair, and the amino acid residues at this site determine the specificity of protease inhibitors (30).

As previously described, serpin B9 plays a role in a number of processes in the body by inhibiting GrB (6). GrB is a caspase-like serine protease released by immune killer cells to kill target cells, such as virus-infected cells and tumour cells (31). Serpin B9, alternatively known as protease inhibitor 9 (PI-9), inhibits multi-pathway killing induced by GrB and exerts a cytoprotective effect (32) (Fig. 3). Cytotoxic T lymphocytes (CTLs) release GrB to injure other cells, while secreted GrB may sometimes be endocytosed by CTLs or accidently released from endosomes (33). In order to protect themselves, CTLs have been revealed to express serpin B9, which protects them from self-inflicted injury (28). Apart from the protective role of serpin B9 in CTLs, it has been demonstrated that serpin B9 exerts the same protective effect on dendritic cells (DC), natural killer (NK) cells and memory cells (34-36). Moreover, studies have indicated that serpin B9 is a marker of antigen cross-presenting DCs and may be involved in the regulation of antigen presentation (37,38). Serpin B9 is widely expressed in various human tissues, including the blood, heart, lung and liver, and particularly in the placenta, testes, ovaries, eyes and other immunocompromised parts, inhibiting immune killing and exerting a cytoprotective effect (39). Thus, it is suggested that serpin B9 principally functions by regulating the immune system.

A previous study reported that serpin B is associated with immune and inflammatory processes in chronic inflammatory diseases, such as atherosclerotic lesions (40). Since then, a large number of studies have focused on the association between serpin B9 and diseases. For example, GrB-positive T lymphocytes have been revealed to cause graft injury by inducing the apoptosis of renal tubular cells (41). However, a high expression of serpin B9 in renal tubular epithelial cells serves as a protective barrier for avoiding acute and chronic rejection (41-43). Moreover, other researchers have demonstrated that serpin B9 is involved in atherosclerosis and diabetes, while circulating serpin B9 mRNA levels have been revealed to be significant and negatively related to the severity of atherosclerotic coronary artery diseases (44). Serpin B9 mRNA expression is also evidently lower in patients with diabetes than in healthy control subjects without diabetes (44). In addition, serpin B9 has been revealed to be involved in the pathophysiological processes of viral infections (45), autoimmune diseases (46), pre-eclampsia (47), cancer (48) and celiac disease (49).

4. Serpin B9 in cancer

In previous years, an increasing number of studies have demonstrated that serpin B9 plays an important role in cancer (50-52).

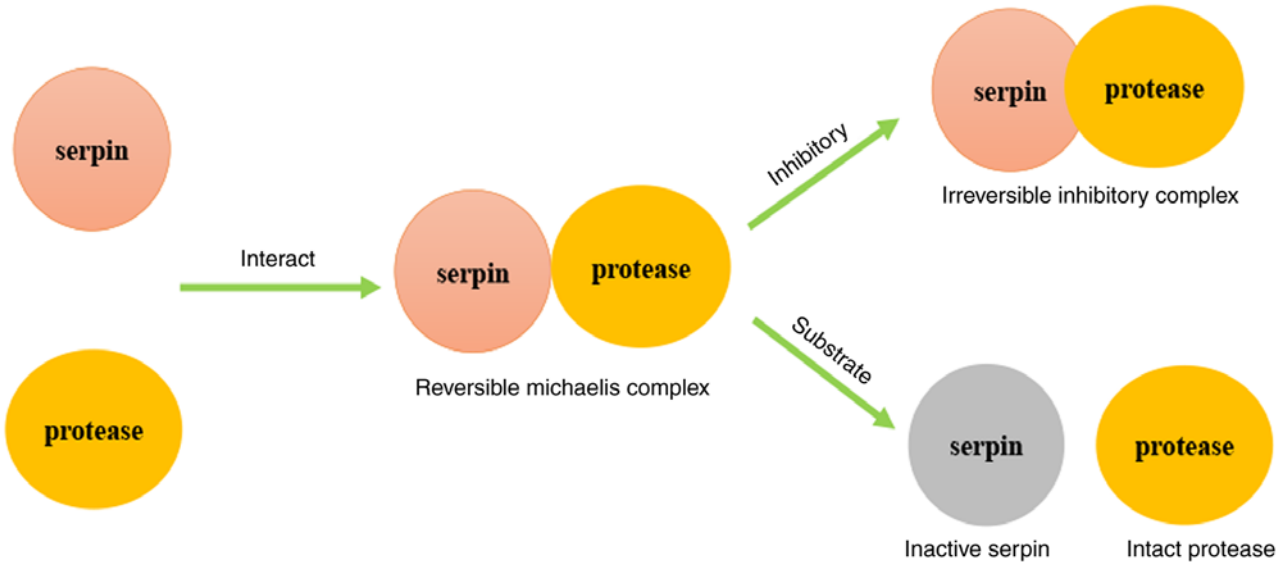

Figure 1. There are two types of serpin inhibitory mechanisms, termed the inhibitory pathway and the substrate pathway. Serpin and protease firstly form a reversible Michaelis complex. According to the SI, the complex proceeds via a different pathway. When SI approaches 1, the irreversible inhibitory complex forms through the inhibitory pathway. When the SI is >1, the cleaved and inactivated serpin and intact protease remain via the substrate pathway. SI, stoichiometry of inhibition.

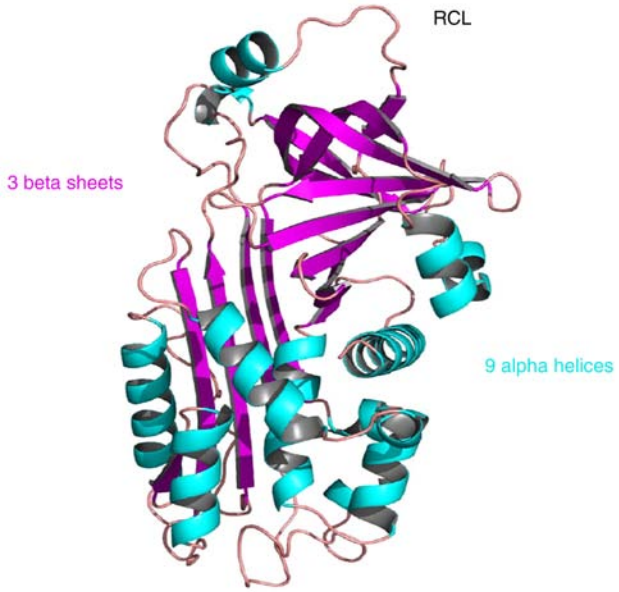

Figure 2. Structure of serpin B9. Serpin B9 has a highly conserved tertiary structure, which includes nine α-helices, three β-sheets, and an RCL at the carboxyl end. Serpin B9, serine proteinase inhibitor B9; RCL, reactive centre loop.

With the increasing research on serpin, the hypothesis that serpin B9 protects tumour cells has emerged (53). According to various studies, the prognostic role of serpin B9 has been described. A high level of serpin B9 was demonstrated to indicate a poor prognosis of patients with uveal melanoma at the genetic and molecular level through bioinformatics methods (54). Another study on 105 patients with hepatocellular carcinoma demonstrated that the expression of serpin B9 in hepatocellular carcinoma was significantly and positively associated with the differentiation, node metastasis stage and size of the tumour (55). Moreover, further Cox regression analysis demonstrated that a high expression of serpin B9 in hepatocellular carcinoma was an independent predictor of poor prognosis and was related to a shorter overall survival (55). Current studies have found that serpin B9 regulates tumour apoptosis, immune evasion, tumorigenesis, metastasis and drug resistance, and may participate in maintaining the stemness of cancer stem cells (6,56-60); thus, it may be a potential novel molecular target for tumour therapy (Fig. 4).

Apoptosis. The association between serpin B9 and the apoptosis of cancer cells has been widely investigated, and varying results have been obtained. Recent research has demonstrated that a GrB-dependent apoptosis of lung cancer cells was observed following the genetic ablation of serpin B9 in lung cancer cells (6). Another study focusing on lung adenocarcinoma presented a similar outcome, in that upregulating the expression of serpin B9 through oestrogen and cigarette side stream smoke particulate matter was accompanied by the promotion of resistance to GrB-mediated apoptosis (61). In patients with non-small cell lung cancer undergoing surgery, the level of serpin B9 mRNA expression was revealed to be upregulated in the less-differentiated lung adenocarcinomas, and primary cells derived from surgical specimens with a higher expression of serpin B9 tended to have lower apoptosis (62). In addition, serpin B9 has been shown to be expressed in ~3/4 of epithelial cancer cell lines and in all leukemic cell lines, and has been confirmed to protect them from apoptosis (63). These aforementioned studies have suggested that serpin B9 inhibits the apoptosis of cancer cells. However, studies on liver cancer have described differential results, which should not be ignored. In a previous study, when upregulating serpin B9 by transient transfection in HepG2 cells, researchers found that a high expression of serpin B9 was associated with decreased apoptosis, which was similar to the findings of previous research (64). Another study used pcDNA3.1-serpin B9 and small interfering (si)RNA-serpin B9

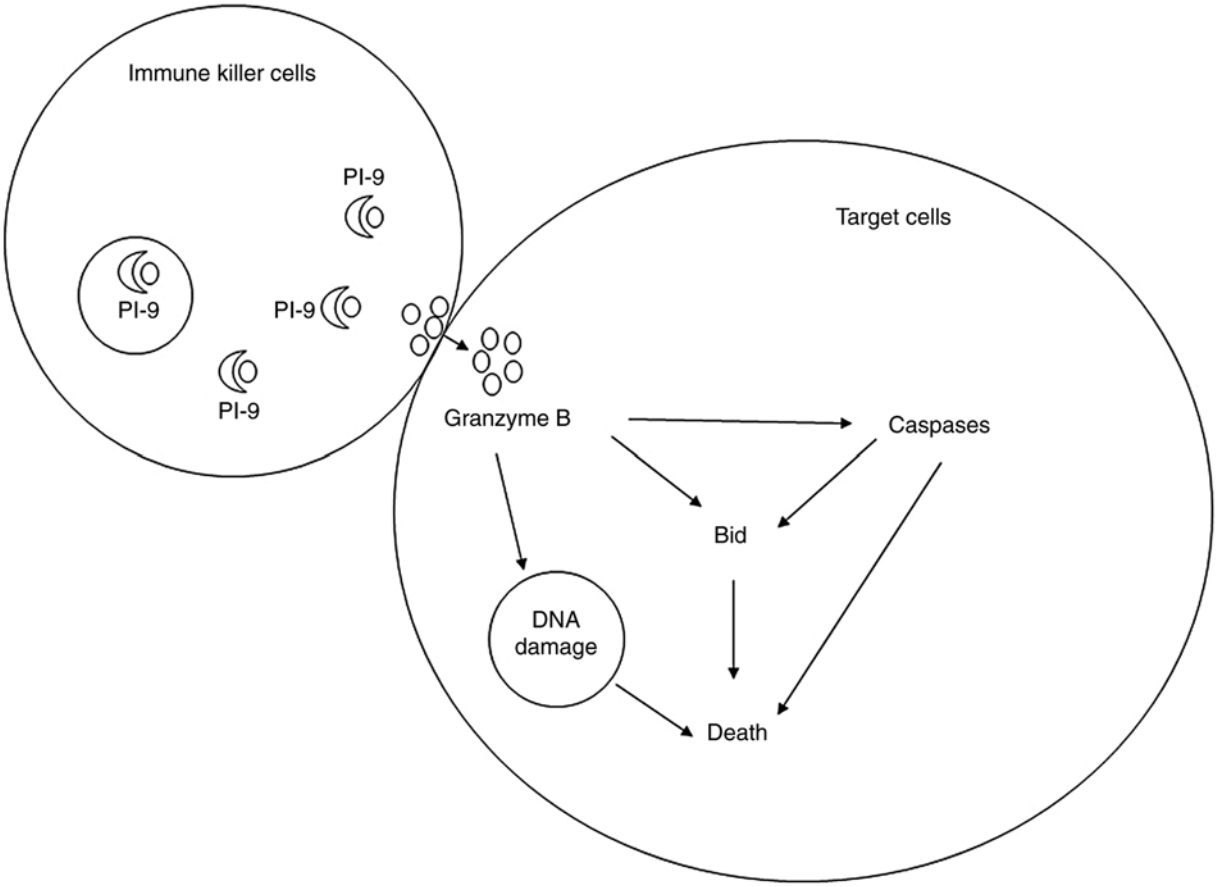

Figure 3. Mechanism of the intracellular serpin B9, PI-9. Granzyme B is produced by immune killer cells and is transmitted to target cells. Serpin B9 inhibits granzyme B activity in the nucleus and cytoplasm of different types of cells, such as cytotoxic lymphocytes, dendritic cells and tumour cells. Serpin B9 protects these cells from death mediated by granzyme B. Serpin B9, serine proteinase inhibitor B9; PI-9, protease inhibitor 9.

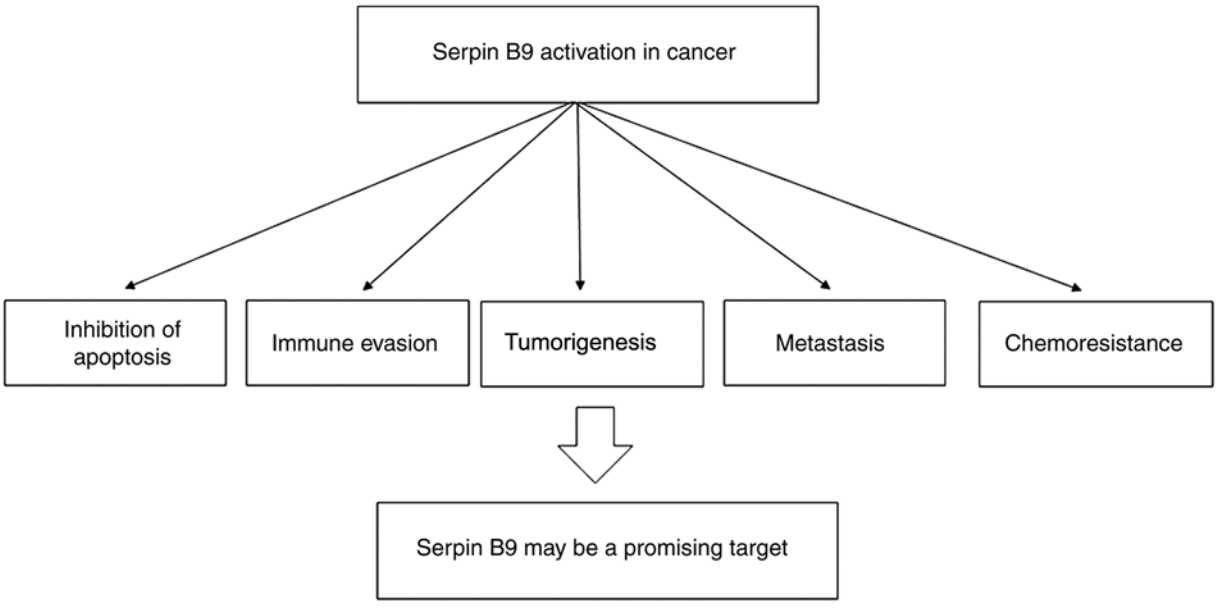

Figure 4. Serpin B9 may be a promising target of cancer. Serpin B9 activation in cancer inhibits apoptosis and promotes the immune escape of cancer cells. In addition, serpin B9 is involved in the development of tumorigenesis, metastasis and chemoresistance in cancer. Serpin B9, serine proteinase inhibitor B9.

to regulate its expression and detected changes in cell proliferation and apoptosis using MTT assay, colony formation assay and flow cytometry, respectively (55). The results of that study demonstrated that the apoptosis of HepG2 cells was markedly decreased following the silencing of serpin B9 using siRNA and was significantly increased following serpin B9

overexpression, which indicated that serpin B9 enhanced the apoptosis of HepG2 cells (55). This result is contradictory to the conclusion that serpin B9 inhibited the apoptosis of cancer cells according to aforementioned studies. However, further Cox regression analysis revealed that serpin B9 expression was an independent factor of poor prognosis of patients with hepatocellular carcinoma (55). The contradictory results in liver cancer have not yet been elucidated, which may be related to specific tumour types. Collectively, the majority of researchers have demonstrated that serpin B9 inhibits the apoptosis of cancer cells; however, further studies are required in order to clarify the role of serpin B9 in different types of cancer.

Apoptotic mechanisms. GrB mediates the apoptosis of cancer cells through a variety of mechanisms, and serpin B9 protects tumour cells mainly by inhibiting GrB (65). GrB induces the death of NK/T-cell lymphoma cells through aspartase-dependent and non-aspartase-dependent mechanisms, which in turn leads to extensive tumour necrosis (66). However, the upregulation of serpin B9 has been revealed to protect tumours by reversing this pathophysiological process (66). In certain tumor necrosis factor (TNF)-sensitive cancer cell lines, serpin B9 has been revealed to inhibit TNF and TNF-related apoptosis-inducing ligand- and Fas ligand-mediated apoptosis (67). Serpin B9 functions as regulator of pro-apoptotic apical caspases (67). In addition to directly inhibiting the apoptotic effect of GrB, serpin B9 has been demonstrated to cooperate with other protease inhibitors to resist the killing effect of GrB. A number of colon carcinoma cell lines have been found to exhibit resistance to membranolysis, which is dependent on serpin B9; however, the expression of an associated serpin known as serine protease inhibitor involved in cytotoxicity inhibition (SPI-CI) is required (68). SPI-CI is a novel immune evasion molecule that functions in association with serpin B9 to protect cancer cells from CTL-mediated death (68). Although the mechanisms involved in serpin B9 are complex, scholars have reached a consensus on the anti-apoptotic effect of serpin B9 on cancer cells.

Immune evasion. The immune system plays a dual role in cancer, acting as a double-edged sword. On the one hand, it prevents cancer cells from undergoing immune killing, while on the other hand, it protects the body with the immune elimination of tumour cells (69). The immune evasion of cancer occurs though the normal host immune surveillance system; tumours still occur, develop, metastasize and invade (4). Aimed at immune evasion, scholars have explored immunotherapy, which has been applied in almost all tumours, such as lung and liver cancer, lymphoma, melanoma and skin cancer (70,71). As aforementioned, serpin B9 participates in the pathophysiological process of diseases by regulating the immune system. Therefore, a number of scholars are interested in the association between serpin B9 and tumour immune evasion. In a previous study describing the expression of serpin B9 *in vivo* in human neoplastic cells, serpin B9 was revealed to be involved in protecting neoplastic cells from immune elimination by CTLs (72). As previously demonstrated, serpin B9 not only protected serpin B9-positive classical Hodgkin lymphoma cells from immune attack by GrB, but also resisted immunotherapy, which was used to enhance the killing effects of GrB, suppressing the immune killing effect or rendering it invalid (56). In order to clarify the association between serpin B9 and immune evasion, a cancer cell line expressing serpin B9 was previously engineered and exhibited an obvious nonexponential survival time distribution, which was confirmed by evidence that two or more immune attacks were likely required to kill serpin B9-expressing cells (73). A similar result was revealed in breast cancer cell line studies. Oestrogen has been shown to induce an increase in the expression of serpin B9 in breast cancer cells, thus preventing immune surveillance, providing a double advantage for breast cancer (74). In addition, in different types of lymphoma, the overexpression of serpin B9 in lymphoma cells has been revealed to trigger an immune disorder in the body and to facilitate the malignant transformation of normal cells (72). Clinical studies have demonstrated that serpin B9 is involved in enhancing immune evasion and this effect is associated with the tumour stage and a poor prognosis as the level of serpin B9 increases (65,75). In addition, there were other studies describing the association between tumour staging and serpin B9. A previous study detected serpin B9 expression in lung cancer cell lines; the study obtained primary lung cancer cells from the curative lung resection of patients with cancer and assessed the influence of conditioned media for lung cancer cell lines on GrB expression and the cytotoxic activity of $CD8^+$ T-cells (65). The results of that study indicated that an increased serpin B9 enhanced the immune evasion of cancer, which was accompanied by the upgrading of the lung cancer stage (65). Moreover, the strong association of serpin B9 with a poor prognosis by promoting immune evasion has been previously demonstrated by the survival analysis of Hodgkin's lymphoma and anaplastic large-cell lymphoma (76). Thus, collectively, there is evidence to indicate that serpin B9 enhances cancer immune evasion.

Tumorigenesis. The association between tumorigenesis and serpin B9 has been investigated by a number of studies. In patients undergoing kidney transplantation, skin squamous cell carcinoma is a severe post-operative complication (77). It has been revealed that, compared with patients without skin squamous cell carcinoma, patients with skin squamous cell carcinoma following transplantation exhibit a significantly increased serpin B9 expression; in addition, a high DNA methylation of serpin B9 circulating T cells has been detected in patients with recurrent cutaneous squamous cell carcinoma (57). These results indicated that a high level of serpin B9 is a high-risk factor of skin squamous cell carcinoma in patients undergoing kidney transplantation (57). A previous study focused on primary malignant brain tumours in children and provided intuitive evidence that serpin B9 is acquired in 29% of cancer during tumorigenesis (78). Similar results have been revealed in placental tissues, which have a low immunity. The serpin B9 expression level in trophoblasts of placental tissues in different gestational stages has been demonstrated to differ significantly (79). Compared with the placenta from early pregnancy, the levels of serpin B9 in trophoblasts of partial and complete hydatidiform moles were significantly higher, and the level of serpin B9 in choriocarcinoma cell lines was the highest (79). These results indicated that serpin B9 expression in trophoblasts was consistent

with the corresponding immune evasion, and the upregulation of serpin B9 expression in gestational trophoblasts may contribute to tumorigenesis. Therefore, serpin B9 may have a potential biomarker role in precancerous lesions (80,81). In patients with prostate cancer, serpin B9 has been identified consistently in high-grade prostatic intraepithelial neoplasia and atrophic lesions, which indicates that early prostatic inflammation may trigger an increase in serpin B9 expression (80). A study on liver cells provided direct evidence. The knockdown of serpin B9 by siRNA blocked the ability of oestrogen, inhibiting cytolytic lymphocyte-mediated immune surveillance to protect newly transformed cells (81). This result provided a novel mechanism for an oestrogen-serpin B9-mediated increase in tumour incidence. Thus, a number of studies have clarified that serpin B9 is intensively involved in tumorigenesis.

Proliferation. At present, only a few studies have focused on the role of serpin B9 in tumour proliferation. In cancer mouse models, when the tumour and host are both deficient in serpin B9, the maximal inhibition of tumour development is detected and serpin B9 inhibition exerts a suppressive effect on tumour growth (6). However, other studies have described conflicting results. In a study on nasal NK/T-cell lymphoma, the expression of serpin B9 was analysed through immunohistochemistry and the overall survival time of patients was followed-up (82). Nasal NK/T-cell lymphoma is a rare Epstein-Barr virus-related malignancy with a poor outcome (83). It has been demonstrated that the absence of serpin B9 and a low apoptotic index are associated with a poor outcome, indicating that a lack of serpin B9 expression in cancer cells may be associated with certain mechanisms related to tumour progression (82). In addition, another study on liver cancer obtained similar results. In HepG2 hepatoblastoma cells, cell proliferation detected using MTT and colony formation assays was decreased following the upregulation of serpin B9, and was markedly increased following the siRNA interference of serpin B9, which suggested that serpin B9 inhibited the progression of liver cancer (55). These studies are contradictory and there is no clear conclusion regarding the role of serpin B9 in the proliferation of cancer.

Metastasis. The majority of malignant tumours cannot be eradicated, and metastasis and recurrence are the main causes of cancer-related mortality (84). According to previous studies, the expression of serpin B9 in tumours tends to promote tumour metastasis. The decreased level of serpin B9 in liver cancer cells is associated with the reduced liver metastasis of colorectal, lung and pancreatic cancer, which indicates that serpin B9 expression in tumours may promote metastasis (85). Another study on lymph node metastasis also supported this conclusion. A total of 152 tumour samples and clinical features from 152 patients who underwent resection for colorectal carcinoma were collected and analysed (58). The results demonstrated that GrB was positively related to a positive outcome of colorectal carcinoma; conversely, the level of serpin B9 indicated lymph node metastasis and a poor prognosis (58). Of note, in the same cancer cell line, serpin B9 appeared to be involved in metastasis to different sites. Compared with breast cancer cell lines which specifically metastasize to other parts, lung-specific metastatic cells have been revealed to significantly overexpress serpin B9 (86). This result provided a new perspective for the investigation of the association between serpin B9 and metastasis. In addition, the findings of the following study should be noted. Specifically, the high expression of serpin B9 in liver NK cells and sinusoidal endothelial cells protected the liver microenvironment from the destruction of the highly active perforin/GrB, maintaining the liver microenvironment stability for killing metastatic cancer cells (87). That study clarified the antitumour effect of serpin B9 in normal tissues. Although serpin B9 in normal tissues was not the main part of the discussion of the present review, the complexity of serpin B9 in tumour metastasis is suggested.

Resistance to chemotherapy. To date, chemoresistance remains the main cause of ineffective tumour treatment (88). Previous studies have demonstrated that serpin B9 is involved in chemoresistance (59,89). A study on locally advanced rectal cancer patients demonstrated that serpin B9 analysed by immunoblotting was negatively associated with the response to neoadjuvant chemotherapy before surgery, indicating that a high level of serpin B9 was related to a poor chemotherapeutic effect (59). In a study on 244 children with acute lymphoblastic leukaemia, compared with patients without minimal residual disease, patients with minimal residual disease had a higher serpin B9 expression (89). The high level of serpin B9 may indicate the chemoresistance of cancer. However, there are a few studies which have demonstrated varying results. Previously, in order to evaluate the role of serpin B9 in cancer, three stably transfected HeLa cell lines expressing wild-type serpin B9 and one cell line expressing the inactive mutant serpin B9 were constructed (90). The results revealed that the high level of serpin B9 in wild-type cell lines protected tumour cells from NK cell-mediated killing; however, it did not influence etoposide-induced apoptosis (90). This result did not support the promoting role of serpin B9 in the chemoresistance of cancer; thus, further more in-depth investigations are warranted to explore the association between serpin B9 and drug resistance.

Radiotherapy. Studies on the role of serpin B9 in tumour radiotherapy are limited. Radiotherapy not only stimulates the immune response to clear tumour cells, but also induces tumour drug resistance (91). The following studies may provide some insight on this matter. In a previous study, after cancer cells were treated with ionizing radiation, serpin B9 expression was upregulated through type I interferon, to protect cancer cells from T cell-mediated cytotoxicity, which suggested that radiotherapy protected cancer cells through serpin B9 (92). Another study conducted on 235 male clean-up workers who were exposed to radiation during the Chernobyl accident in 1986-1987 indicated that the expression of serpin B9 was decreased by radiation in a dose-dependent manner, which suggested that radiation may reduce the tumour protective effects of serpin B9 (93). These results were contradictory, although they indicated the 'double-edged sword' effect of radiation. However, to date, to the best of our knowledge,

there is no study available exploring the association between serpin B9 and radioresistance, which may become a future research hotspot.

Immunotherapy. Given the regulatory role of serpin B9 in the cancer immune system, a number of studies have focused on the association between immunotherapy and serpin B9. According to the expression of serpin B9 in various human and murine tumours, and its role in resistance to CTL-mediated killing with the inhibition of the perforin/GrB pathway, Medema *et al* (94) identified that serpin B9 was an important parameter determining the success of T-cell-based immunotherapeutic modalities aimed at solid cancer. Moreover, research on classical Hodgkin lymphoma has demonstrated similar findings, in that the overexpression of serpin B9 was described in the most classical Hodgkin lymphoma-derived cell lines to protect them against the GrB attack for resisting GrB-based immunotherapies (56). The 'double-edged sword' effect of the immune system in cancer is presented in the association between serpin B9 and immunotherapies. As previously demonstrated, in oesophageal cancer and gastric cancer cells, when the expression level of serpin B9 is decreased, the apoptosis of invariant NK T (iNKT) cells is increased and the effect of iNKT cell-based immunotherapies is decreased, which suggests that serpin B9 enhances the effects of iNKT cell immunotherapies (95). In addition, the expression of serpin B9 in a specific site has been revealed to cooperate with another immunotherapy for an enhancing effect. At present, genetically modified DCs are regarded as a promising immunotherapy for the treatment of cancer (96). Through introducing serpin B9 expression vectors into embryonic stem (ES) cells and subsequently inducing differentiation to DCs, the genetic modification of ES-DCs overexpressing serpin B9 enhances the capacity to prime antigen-specific CTL in semi-allogeneic recipient mice to assist immunotherapy (97). Thus, the expression of serpin B9 in different sites exerts varying protective or killing effects on cancer, based on the cytoprotective effect of serpin B9. For this reason, strategies aimed at maximizing the killing effect on tumours by regulating the level and expression sites of serpin B9 may become future study hotpots.

Cancer stem cells (CSCs). CSCs are a small part of immature cells in tumours, which have a strong capacity of self-renewal, multidirectional differentiation and infinite proliferation, and are intensively involved in the tumorigenesis, development, metastasis and drug resistance of cancer (98,99). Therefore, it is meaningful to study the association between serpin B9 and CSCs. Studies have revealed that bone mesenchymal stem cells and embryonic stem cells highly express serpin B9, which protects them from the killing effect of GrB released by CTLs (100,101). This indicates that serpin B9 protects normal stem cells from apoptosis; however, there are a few studies focusing on whether serpin B9 participates in maintaining the stemness of stem cells. Studies on serpin B9 in CSCs provide some insight. In tertiary breast cancer cell spheres, which exhibit high levels of stemness markers (Nanog, Oct3/4 and Sox2) and a self-renewal capacity, high levels of C-X-C motif chemokine receptor (CXCR)4 and phosphorylated p38 have been detected (60). CXCR4 is a specific receptor for C-X-C motif chemokine ligand 12 (CXCL12), which has been identified to be involved in maintaining the stemness of CSCs and to participate in the chemotaxis of CSCs (102). Another study demonstrated that activating the CXCR4/phosphorylated p38 axis increased the level of serpin B9 for interfering with the immune surveillance system, which enhanced the proliferation and self-renewal ability of breast cancer stem cells (60). In addition, Hjelmeland *et al* (103) demonstrated that the expression of hypoxia inducible factor (HIF)-2α in glioma was significantly upregulated in the acidic tumour microenvironment, and serpin B9, as a specific target gene of HIF-2α, not only directly interacted with the target gene promoter, but also promoted the transcription of important stem cell factors through epigenetic modification in brain glial stem cells. This indicated that serpin B9 plays an important role in maintaining the stemness of CSCs, providing a novel target for future targeted CSC therapy.

5. Conclusion and future perspectives

Studies have indicated that serpin B9, as a serine protease inhibitor, mainly inhibits the immune killing effect of GrB to protect tumour cells. According to numerous studies, the expression of serpin B9 in tumours decreases apoptosis, enhances immune evasion and promotes tumorigenesis, metastasis and chemoresistance, and is even involved in maintaining the stemness of CSCs. In addition, clinical studies have demonstrated that a high level of serpin B9 was associated with the occurrence and a poor prognosis of cancer, which could possibly become a predictor of ineffective chemotherapy and immunotherapy. Collectively, serpin B9 may be a potential novel biomarker and target for tumour therapy.

However, a number of issues regarding serpin B9 remain to be addressed. Firstly, in terms of tumour proliferation and radiotherapy, the results of current research are inconclusive and even contradictory. Secondly, to the best of our knowledge, there is no study available focusing on the association between tumour angiogenesis and serpin B9. Thirdly, although several studies have indicated the protective role of serpin B9 in cancer, the exact mechanisms remain elusive due to the lack of comprehensive research, particularly in terms of CSCs. Fourthly, the influence of serpin B9 expression in normal tissues on tumours remains unclear. Finally, the strategy of targeting serpin B9 to enhance the tumour-killing effect of chemotherapy or immunotherapy remains indistinct and unstable. Therefore, further in-depth studies are required to clarify the association between serpin B9 and tumours.

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Availability of data and materials

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Authors' contributions

WJW, JW and XQY developed and designed the idea and wrote the manuscript. CC, XFX, CO, JW and WJW performed the literature review and graphing. All authors read and approved the final manuscript.

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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