Antitumor Effects and Mechanisms of Snake Venom: A Systematic Review

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Abstract: Snake venom, as a natural biotoxin, is widely present in nature and can act in coordination with a variety of signal regulatory proteins, playing a significant role in the regulation of tumor cell proliferation, metastasis, invasion, and angiogenesis. Malignant tumors have long attracted the attention of the medical community as the leading cause of death in humans. In this paper, we focus on reviewing the progress of snake venom in the regulation of apoptosis, proliferation, metastasis, invasion, and angiogenesis of tumor cells in malignant tumors in order to clarify the mechanisms by which snake venom suppresses malignant tumors and to provide a reference for the study of malignant tumors.

1 INTRODUCTION

With the effective control of severe infectious diseases and the extension of human life expectancy, cancer has become one of the major diseases that seriously endanger human health. Currently, cancer is one of the top two causes of death among middle-aged and older residents in most countries. Annual new cancer cases worldwide are projected to increase by 50% from 18 million in 2018 to 27 million in 2040. Among them, the growth of new cancer cases in developing countries is higher than that in developed countries (Deng 2020). Because most cancers are diagnosed too late and prevention measures are inadequate, cancer is becoming a heavy burden for people in low - and middle-income countries (Xu 2020).

Current anti-tumor treatments mainly include surgical treatment, chemotherapy, radiotherapy, immunotherapy and traditional Chinese medicine treatment, etc.. Radiotherapy and chemotherapy are the main methods for the treatment of malignant tumors (Xue 2020, Zhang 2020, Chen,2020). Although there are many clinical antitumor drugs, such as alkylating agent class of drugs, drug metabolism, antitumor antibiotics, antitumor hormonal drugs, metal platinum, antitumor medicine plants, molecular targeted drugs, etc., but the effect of uncertainty and high resistance, high cost, so the treatment of the tumor remains very difficult (NIELSEN 2016).

Snake venom is a protein secreted from the venom glands of venomous snakes and contains a variety of enzymes. Its main components include neurotoxins, cardiotoxins, cytotoxins, bleeding toxins, procoagulant and anticoagulant components, etc (Liu 2019). With the development of biochemistry and molecular biology techniques, the clinical applications of snake venom have received increasing attention. Over the years, scientists from various countries have continuously explored the neighborhood of snake venom anti-tumor and made some achievements (Li 2019). Snake venom can inhibit the growth of tumor cells by directly killing tumor cells, inducing tumor cell apoptosis, and inhibiting angiogenesis (Jin 2015). In this paper, we summarize and analyze relevant recent national and international research results to provide a reference for the study of snake venom against tumors.

2 THE EFFECT OF SNAKE VENOM ON TUMOR CELL PROLIFERATION

One of the main characteristics of tumor cells is their unlimited proliferation. By inhibiting the proliferation of tumor cells, the malignant growth of the tumor can be controlled and the damage to the body can be reduced.

Thien V Tran et al. isolated phospholipase A2, a cytotoxic protein, from the venom of Bungarus multicinctus. MTT assay was used to detect the effect of the protein on the proliferation of MCF7 and A549 cancer cells, and the results showed that the protein had a dose - and time-dependent cytotoxicity on MCF7 and A549 cells, but had no toxic effect on human normal kidney HK2 cells (Tran 2019). Tian Dahao et al., isolated the tumor inhibitory component (AHVAC-1) from Deinagkistrodon acutus venom, and tested the inhibitory effect of different concentrations of AHVAC-I on the proliferation of SGC-7901 cells by MTT method in vitro (Tian 2020). The results showed that: AHVAC-I inhibited the proliferation of SGC-7901 cells in a dose-dependent manner within a certain range (3-30 mg/L). When Xiong Yan et al. studied the venom of Deinagkistrodon acutus venom, they found that different concentrations and different times of Deinagkistrodon acutus venom had inhibitory effect on ovarian cancer

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A2780 cells, and it was time and concentration dependent (Xiong 2019).

Some snake venom proteins or peptides, such as lectins, snake venom cys-eobatin (sv-cystatin), L-amino acid oxidase (NA-LAAO) and so on, can inhibit the proliferation of tumor cells and block the tumor cell cycle. Manel B. Hammouda et al. found that the C-type lectin (Macrovipecetin) in the venom of Macrovipera lebetina had a significant inhibitory effect on the proliferation of melanoma SK-MEL-28 in a time - and dose-dependent manner (Manel 2018).

3 THE EFFECT OF SNAKE VENOM ON APOPTOSIS OF TUMOR CELLS

Apoptosis is a genetically regulated physiological and pathological process that occurs in all cells in response to death signals and is a Programmed cell death (PCD) mode. Apoptosis affects the occurrence and development of diseases in the body. Apoptotic cells shrink and condense, the cytoskeleton collapses, the nuclear membrane is dismantled, and the nuclear DNA is decomposed into segments (Alluzzi 2015). Malignant tumor cells are characterized by shutting down the programmed death pathway, causing disordered cell growth and damaging the organism. Therefore, finding drugs that induce apoptosis in tumor cells is one of the open directions of modern anti-tumor drugs.

3.1 Induction of tumor cell apoptosis by activation of the mitochondrial death pathway

Mitochondria are organelles that provide energy to the cell and are one of the key organelles in the apoptosis process. It has been found that snake venom can induce mitochondrial dysfunction, leading to apoptosis of tumor cells.

Zhao Rong et al. found that tumor suppressor component I (AHVAC-I) of Agkistrodon Wannan Pallas (AHVAC-I) could cause the death of SGC-7901 cells accompanied by typical apoptotic morphological characteristics such as chromatin aggregation, nuclear fragmentation and apoptotic bodies. The decrease of mitochondrial membrane potential ($\triangle\Psi m$) was detected by chemical fluorescence assay, suggesting that the mechanism of AHVAC-I on the apoptosis of SGC-7901 cells was the change of mitochondrial membrane permeability and the release of apoptotic proteins (Zhao 2018).

Xu Cheng et al. isolated a tumor suppressor component I (AAVC-1) from the venom of Deinagkistrodon acutus in southern Anhui. In this study, AAVC-1 was shown to induce apoptosis in the human non-small cell lung cancer cell line A549. The mitochondrial membrane potential was detected by Jc1, and the distribution of cytochrome C in mitochondria was detected by immunohistochemistry. The results showed that the percentage of green fluorescence of mitochondrial membrane potential and the mean optical density of cytochrome C in the cytoplasm increased significantly with the increase of AAVC-1 concentration, indicating that AAVC-1 could activate mitochondrial pathway to induce apoptosis of human non-small cell lung cancer A549 by reducing mitochondrial membrane potential and promoting the release of cytochrome C (Xu 2017).

Attarde et al., in their study of Cobra NN-32 toxin, found that NN-32 showed an inhibitory effect on breast cancer lines McF-7 (ER+) and MDA-MB-231(ER-), while the effect on normal breast cells was relatively reduced. The main cancer-fighting mechanism of NN-32 may be the mitochondrion-dependent cell death pathway, which up-regulates the expression of the proapoptotic protein Bax and down-regulates the expression of the antiapoptotic protein Bcl-2, resulting in the activation of caspase 3 and 9, leading to programmed cell apoptosis (Attarde 2017).

3.2 Endoplasmic reticulum stress promotes tumor apoptosis

Endoplasmic reticulum (ER) is an important organelle in cells. When cells are stimulated, it will lead to changes in ER calcium homeostasis and protein unfolded or misfolded and accumulation in the ER, leading to ER stress (Li 2019). ER stress promotes the processing of misfolded or unfolded proteins in the lumen of the reticulum, thus better maintaining the normal function of cells and allowing them to survive. However, persistent severe ER stress can induce cell apoptosis (Liao 2018).

AAVC-I was found to induce apoptosis in human tongue squamous cell carcinoma Tca8113. The expression levels of GRP78, CHOP, Caspase-12, Caspase-9 and Caspase-3 were up-regulated by Western blotting. These results indicated that AAVC-I induced apoptosis of human tongue squamous cell carcinoma Tca8113 cells through ER stress CHOP/Caspase-12 pathway (Chai 2020).

3.3 Promote apoptosis of tumor cells through autophagy

Autophagy is a lysosomal-dependent degradation pathway. It is an ubiquitous life phenomenon and an important way of cell degradation (Kacper 2021, Aileen 2021, Li 2020). Autophagy is a process that relies on lysosomes to degrade macromolecules and some organelles for reuse. Basal levels of autophagy have a protective effect on cells, but high levels of autophagy can also excessively degrade organelles and promote apoptosis.

Tingting Huang et al. found that AAVC-I may up-regulate expression levels of Caspase3 through certain pathways in a concentration-dependent manner. The results of the JC-1 assay indicate that the expression level of Caspase 3 may be related to changes in the membrane potential. At the same time, the expression of LC3 was increased by immunofluorescence, indicating that AAVC-I could improve the autophagy level of oral squamous cell carcinoma HN4 cells, suggesting that the pro-apoptotic effect of AAVC-I on oral squamous cell carcinoma cells may increase the expression of Caspase-

3 by reducing mitochondrial membrane potential and increase the level of autophagy (Huang 2020).

4 THE EFFECT OF SNAKE VENOM ON TUMOR CELL MIGRATION AND INVASION

The main characteristics of tumor cells are invasion and metastasis. The mechanism of tumor cell invasion and metastasis is complex and involves the interaction of many regulatory factors, cytokines, etc., and is the main cause of malignant tumor development. Changes in the ability of cells to adhere to each other are the initial stage of cell metastasis and contribute to the shedding of tumor cells from the primary site, the invasion of surrounding tissue, and the initiation of the metastasis program. Cancer cell invasion and metastasis are still important causes of death.

agkihpin is an arginine esterase purified from the venom of the Agkistrodon halys pallas. It can inhibit the migration of human hepatocellular carcinoma cells and nasopharyngeal carcinoma cells. After treatment of the HCC cell line with different concentrations of agkihpin, tumor cell viability was detected by MTT assay, and the effect of acrimony on cell migration was detected by cell counting. The results showed that agkihpin significantly inhibited the migration of human liver SMMC-7721 cells with an inhibition rate of 7.8%-98.0%, and the higher the dose, the more obvious the inhibitory effect (Hu 2012).

Chen Liu used Chinese Cobra venom membrane toxin-12 (MT-12) and found that MT-12 inhibited the migration and adhesion ability of bladder cancer RT4 and T24 cell lines through flow cytometry, cell scratch and cell adhesion experiments (Chen 2018). Akbar Oghalaie et al. first evaluated the cytotoxicity and anti-adhesion effect of V.betinaturanica venom and its components on lung epithelial tumor cells (TC-1), and the results proved that it had an anti-adhesion effect on lung cancer cell line TC-1, which is expected to become a new treatment method (Oghalaie 2017).

Most snake venom disintegrins are cysteine rich, have a molecular weight of about 5-9 kDa, and are typically characterized by the presence of RGD (Arg-Gly-Asp) or KGD (Lys-Gly-Asp). Most integrins recognize RGD sequences and most extracellular matrices contain RGD sequences, so RGD is an important site for extracellular matrix binding to cellular integrins. Disintegrins play a role in tumor inhibition by competing with integrin receptors on the cell surface to block the adhesion of tumor cells to the extracellular matrix. Meng Longlong used the recombinant protein rAdinbitor obtained from the venom gland of Agkidon pallipallas to detect the proliferation, adhesion, migration and invasion capabilities of mouse liver cancer Hca-P cells by Transwell chamber assay. The results showed that: rAdinbitor can regulate the proliferation, adhesion, migration and invasion of mouse hepatoma Hca-P cells through Crki-mediated signaling pathway (Meng 2016).

Endothelial to mesenchymal transition (EMT) is a lineage transformation process between epithelial cells and mesenchymal cells, in which polarized epithelial cells lose adhesion and acquire mesenchymal phenotype (Chen 2017). This process is an important link in human embryonic development, while EMT promotes disease progression and enhances the metastatic phenotype by conferring previously benign cancer cell characteristics such as migration, invasion, chemoresistance and tumor initiation potential (Chaffer 2016). Huang Miao et al., through real-time quantitative PCR, Western blot, bioinformatics technology and enzyme activity analysis, found that agkihpin up-regulated the expression of epithelial cell marker E-cadherin in hepatocellular carcinoma SMMC-7721 and Hep G2 cells (Huang 2016). Downregulated expression of the mesenchymal markers N-cadherin and Vimentin, as well as the transcriptional regulators Snail and twist. agkihpin down-regulates the expression of FZD7 and β-catenin, reduces the phosphorylation of GSK3β (Ser9) and nuclear deposition of β-catenin, and inhibits the Wnt/β-catenin pathway in hepatoma cells. These results suggest that agkihpin inhibits the metastasis and invasion of hepatocellular carcinoma cells by mediating epithelial-mesenchymal transition through Wnt/β-catenin signaling pathway.

5 THE EFFECT OF SNAKE VENOM ON TUMOR ANGIOGENESIS

Tumor angiogenesis is a key step in tumor growth, invasion and metastasis, and it is also one of the research fields of tumor targeted therapy. Inhibition of tumor angiogenesis has been recognized as an effective anticancer strategy (Kyoko 2018).

Bothro-eobidin, a component isolated from Bothrops pauloensis snake venom, reduced endothelial cell viability and adhesion and inhibited basic fibroblast growth factor-induced angiogenesis in vitro. It is suggested that bothro-eob-poidin has an antiangiogenic effect (Guimarães 2017).

Different venom components inhibit the invasion and migration of endothelial cells by different mechanisms. The anti-angiogenic activity of snake venom disintegrin is related to the inhibition of VEGF expression in cancer cells by blocking the Akt and ERK1/2 signaling pathways. Zakraoui O et al. found that Lebein significantly reduced the expression of VEGF and neuropilin-1 in LS174 cells by inducing activation of the reactive oxygen species-triggered protein kinase 1/2 pathway. In addition, Lebein reduced LS174 cell adhesion and migration by down-regulating integrin α5β1 expression (Zakraoui 2017). Ji Mingkai et al used the chick embryo chorioallantoic membrane angiogenesis model to observe the effect and mechanism of SVMPI recombinant protein isolated from the serum of Agkistrodon American on angiogenesis (Ji 2017). It has been tentatively demonstrated that SVMPI recombinant proteins may block the VEGF-KDR or B-type FGF-FGFR signaling pathways. It can inhibit angiogenesis and provides theoretical basis and experimental data for the development of SVMPI recombinant protein as an anti-tumor drug. Snake venom disintegrin lebein inhibits VEGF-induced neovascularization in the CAM system of

quail embryos and blocks the occurrence of human colon adenocarcinoma in nude mice (Zakraoui 2017).

6 CONCLUSIONS

Snake venom is a natural animal toxin and is an important area of research in natural animal toxins. Snake venoms have been found to have antitumor, procoagulant, and anticoagulant effects. Not only is it the most concentrated natural reservoir of enzyme components in nature, it is also an important resource for the potential discovery of new bioactive molecules. However, from available studies, snake venoms have a variety of potential physiological functions and medicinal value. Currently, drugs used in clinical practice are primarily drugs that act on the blood system. Studies have shown that snake venom plays an irreplaceable role in basic theory and application research as a molecular template for new biomedicine, a tool reagent for neurobiology research, a leading molecule for new biological insecticides, and a framework molecule for protein engineering (Zhang 2017).

In today's society, cancer remains a major risk factor that threatens human life and is a focus of medical attention. The anti-cancer effects of snake venom in cancer are associated with regulation of expression of key proteins involved in tumor cell proliferation, apoptosis and migration, regulation of epithelial-mesenchymal transition, activation of mitochondrial death pathway, endoplasmic reticulum stress, cell autophagy, and inhibition of tumor angiogenesis. However, the specific mechanism is still a research hot spot. With the development of molecular biotechnology, recombinant snake venom proteins with high activity and purity are expected to be produced through molecular cloning techniques. Meanwhile, in anti-tumor studies, most active components of snake venom have targeted damage to tumor cells but no cytotoxicity to normal human cells, which has great potential to be targeted anti-tumor drugs.

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