

Analysis of the Mechanism and Therapeutic Effect of Clematis chinensis in the Treatment of Oral Cancer

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Abstract. The incidence of oral squamous cell carcinoma (OSCC) is showing a trend of affecting younger people. Traditional surgery, radiotherapy, and chemotherapy have limitations, such as postoperative dysfunction, drug resistance, and a high recurrence rate. The saponins, flavonoids, and polysaccharides are active components of *Clematis chinensis* that can inhibit oral cancer through multiple mechanisms in a synergistic manner. Clinical compound combinations can enhance efficacy and reduce toxicity, but potential hepatotoxicity should be monitored. At present, there are deficiencies in the research on its targeted improvement and the combination strategy with traditional radiotherapy and chemotherapy. This article specifically analyzed the mechanism of action of the active ingredients of *Clema chinensis*, the effect of compound application and potential toxicity. The results showed that its active ingredients have the advantages of multi-target and multi-pathway inhibition. Combined application can enhance the therapeutic effect and reduce some toxicity, but the risk of liver toxicity needs to be paid special attention to.

Keywords: *Clematis chinensis*, active ingredients, anti-tumor mechanism, combination therapy, oral squamous cell carcinoma.

1. Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor in the head and neck region. The global incidence rate shows significant regional differences, with a particularly high incidence in developing countries. The five-year survival rate is only 40-50% [1, 2]. OSCC often presents as ulcerative lesions of the oral mucosa, involving the tongue and chewing functions, leading to articulation disorders, dysphagia, etc., which seriously affect the quality of life of patients [3]. Although traditional treatments (surgery, radiotherapy and chemotherapy) are the main approaches, postoperative functional impairment, drug resistance and recurrence risk limit the therapeutic effect [1].

In recent years, the unique value of traditional Chinese medicine (TCM) in the treatment of tumors has become increasingly prominent. TCM compound prescriptions and their active ingredients have demonstrated significant auxiliary therapeutic effects through synergistic anti-tumor, regulation of the immune microenvironment, and reduction of the toxic and side effects of radiotherapy and chemotherapy [4]. *Radix Clematidis*, derived from the dried rhizomes of *Clematis chinensis* and other plants of the Ranunculaceae family, was first recorded in "Kai Bao Ben Cao"

and was named for its "combination of vigor and virtue" [5]. According to TCM, it is pungent and warm in nature, belongs to the bladder meridian, and is good at dispelling wind and dampness, unblocking meridians and relieving pain. Modern pharmacological research has confirmed that it has anti-inflammatory, anti-tumor and immunomodulatory effects. Triterpene saponins (represented by clementine C) can induce apoptosis of breast cancer, liver cancer and other cells, reduce tumor angiogenesis, and also inhibit inflammation and enhance the activity of immune cells. Oleanolic acid can block the cell cycle of colon cancer cells to inhibit proliferation, promote the function of immune cells, and enhance the efficacy of cisplatin. Flavonoids (such as quercetin) can alleviate the damage caused by radiotherapy and chemotherapy, activate tumor suppressor genes, regulate immune balance, and work together to exert anti-tumor effects [2, 3].

Clepsyllum has demonstrated clear anti-tumor activity in both breast cancer and liver cancer studies. Regarding breast cancer, clepsyllum saponin C can act on MCF-7 cells, significantly altering the ratio and activity of related proteins by regulating the mitochondrial apoptotic pathway. At a concentration of 10 μ M, the apoptotic rate reaches 38.6%. The polysaccharides of *Clematis chinensis* with specific molecular weights can regulate the expression of related genes and proteins, and the inhibition rate of MCF-7 cell proliferation within 48 hours reaches 52.3%. In animal experiments, nude mice were intraperitoneally injected with total saponins of *Clematis chinensis* for 21 days, resulting in a 47.6% reduction in tumor volume and significant improvement in immune-related indicators.

In terms of liver cancer, 15 μ M of pulpulin acting on HepG2 cells can reduce mitochondrial membrane potential and increase reactive oxygen species levels, with a 24-hour apoptosis rate of 41.2%. Bel7402 cells were treated with 100 μ g/mL of acacia polysaccharides for 48 hours, which could regulate the expression of related proteins, and the apoptosis rate reached 38.9%. In animal experiments, mice were gavaged with the raw extract of *Clematis chinensis* for 14 days, resulting in a 51.3% reduction in tumor weight, a decrease in the number of lung metastases, and an increase in immune cell infiltration [2].

However, *Clematis* has significant limitations in the research of OSCC. There is a lack of empirical evidence on the regulation of specific targets and characteristic pathways of OSCC by its core components, and the influence of the tumor microenvironment (TME) has not been clarified. Animal experiments lack dose gradient and lymphatic metastasis monitoring, and the administration method is divorced from clinical practice. There is a lack of clinical controlled trials, and the data on mucosal tolerance and the synergistic effect with standard treatment are blank [3].

This article systematically reviews the active ingredients of *Clematis chinensis* and its anti-OSCC mechanism, explores its clinical value and future development direction, and provides a theoretical basis for new targeted therapies.

2. Pathogenesis of oral cancer

The pathogenesis of OSCC is essentially the combined result of genomic abnormalities, dysregulation of signaling pathways, metabolic reprogramming and the interaction of environmental factors [6].

At the molecular level, oncogenes such as rat sarcoma virus oncogene homolog (KRAS) continuously activate the MAPK signaling axis through high-frequency hotspot mutations (G12D/V) to drive abnormal cell proliferation, while amplification of myeloblastoma virus oncogene homolog (MYC) gene leads to overexpression of Cyclin D1, accelerating the G1/S phase transition. Correspondingly, the tumor suppressor gene TP53 loses its DNA damage repair function due to inactivated mutations such as R175H/R248W, while the CDKN2A/p16 gene loses control of

the RB-E2F pathway due to hypermethylation of the promoter [3]. It is worth noting that the incidence of TP53 mutations in OSCC is as high as 80%. These two key types of mutations jointly disrupt cell cycle checkpoints and apoptotic mechanisms, leading to the continuous accumulation of mutations and promoting tumor progression.

At the signal pathway level, the abnormal activation of the PI3K-AKT-mTOR pathway is the core driving force for the occurrence and development of OSCC. Among them, the functional acquisition mutation of PIK3CA (E542K/H1047R) or the deletion and excessive activation of PTEN protein enhance survival signals by phosphorylating the pro-apoptotic proteins BAD and FOXO1, and activate the mTORC1 complex to upregulate the expression of HIF-1 α , ultimately driving tumor invasion and metastasis [7, 8].

At the metabolic level, it presents typical aerobic glycolysis (Warburg effect) characteristics, characterized by the overexpression of key enzymes HK2, PKM2 and LDHA under the regulation of oncogenes, which forces a large amount of glucose to be converted into lactic acid. At the same time, the efficiency of mitochondrial oxidative phosphorylation decreases, causing metabolic intermediates to be redirected to biosynthetic pathways to meet the needs of tumor proliferation [9]. The lactic acid accumulation generated by this process further acidifies the microenvironment and induces an immunosuppressive state by activating the GPR81 receptor [7, 9].

In terms of environmental risk factors, tobacco polycyclic aromatic hydrocarbons (benzo [a] pyrene) and acetaldehyde, a metabolite of ethanol, directly induce mitochondrial genomic damage by forming DNA adducts. The synergistic effect of the two increases the risk of OSCC by 5.6 times [10]. Chronic mechanical stimulation (such as poor restorations or sharp tooth tips) induces an IL-6/TNF- α cytokine storm by activating the NF- κ B pathway, promoting the transformation of precancerous lesions [3]. The gingival protease secreted by oral pathogenic microorganisms such as *P. Gingivalis* can specifically degrade the p53 protein, while *Candida albicans* catalyzes the generation of nitrosamine carcinogens. In addition, nutritional deficiencies also significantly increase the risk of disease. Vitamin A deficiency leads to abnormal differentiation and hyperkeratosis of epithelial cells, while insufficient zinc affects the function of zinc finger proteins related to DNA damage repair. Together with oxidative stress damage, they weaken the self-repair ability of the mucosal barrier [1, 3]. It is notable that these modifiable external factors contribute approximately 90% of the disease burden of OSCC in developing countries, highlighting the central role of prevention strategies in reducing morbidity.

3. The therapeutic mechanism of *Clematis chinensis*

The chemical components of *Clematis chinense* include triterpene saponins, polysaccharides, alkaloids and flavonoids, among which oleanolic acid of the triterpene saponins is the main active component [11]. Among them, triterpene saponins exhibit anti-inflammatory and anti-tumor activities by inhibiting the NF- κ B pathway and regulating lipid metabolism [11]. Polysaccharide components induce G0/G1 phase arrest and further induce apoptosis in cell lines such as tongue squamous cell carcinoma CAL-27 [11]. Alkaloids and flavonoids exert analgesic and anti-inflammatory effects by inhibiting the release of inflammatory mediators [11].

3.1. Oleanolic acid

Oleanolic acid is a triterpene component with a relatively high content in *Clepsylla* (the Chinese Pharmacopoeia stipulates that its content is $\geq 0.30\%$). Its chemical structure belongs to the oleanane-type pentacyclic triterpene, and it contains active groups such as carboxyl and hydroxyl groups in its

molecule. Its structural stability and lipophilicity enable it to efficiently penetrate cell membranes and exert its functions.

Oleanolic acid has multi-dimensional activity. In terms of anti-tumor, colon cancer cells (HT-29) were blocked at the G0/G1 phase, Cyclin D1 and CDK4 were down-regulated to inhibit proliferation, and the inhibition rate of HT-29 was 45.6% after 48 hours (CCK-8 method). It can also promote the maturation of dendritic cells and antigen presentation, increasing the infiltration of CD8⁺T cells in tumor tissues of tumor-bearing mice by approximately 30% (immunohistochemistry). In terms of anti-inflammation, it inhibits the NF- κ B pathway, reduces the release of IL-6 and TNF- α , and at 10 μ M, it lowers the IL-6 secretion of lipopolysaccharide-induced macrophages by 52.3% (ELISA). In addition, in combination with cisplatin, it inhibits the NF- κ B pathway to reverse drug resistance, reducing the IC₅₀ of cisplatin on liver cancer HepG2 cells by 38.7% [12].

Numerous studies have confirmed that oleanolic acid, with its structural characteristics, plays a significant role in tumor adjuvant therapy and inflammation-related diseases by regulating the cell cycle, immune microenvironment and inflammatory pathways. It is a representative component among the active ingredients of *Clematis chinensis* that possesses both research depth and application potential.

3.2. Flavonoids

Flavonoids are an important class of active components in *Acacia chinensis*, mainly including quercetin, kaolin, luteolin, etc. They all have benzopyranone as the parent nucleus structure. The substitution of hydroxyl groups at different sites endows them with different activity intensities, and they have strong antioxidant and liposoluble properties, which are convenient for them to pass through the cell membrane and exert their effects. In terms of extraction and analysis, ultrasonic extraction with 50% ethanol is usually adopted. After purification by polyamide column chromatography, quantitative analysis is conducted through high-performance liquid chromatography (for example, the characteristic absorption peak of quercetin is located at 370nm). Combined with mass spectrometry, the molecular weight and glycosidic bond connection mode can be clearly determined, providing a basis for component identification [13].

Its main mechanisms of action are anti-inflammatory, analgesic and immune regulation. In terms of anti-inflammation, quercetin competitively binds to cyclooxygenase-2, reducing the synthesis of prostaglandin E₂ (by 47% in lipopolysaccharide-induced macrophages), and down-regulates the Toll-like receptor 4/MyD88 pathway, reducing the release of nitric oxide by 52%. In terms of analgesia, instillation of 20mg/kg quercetin reduced the number of acetic acid twists in mice by 60%, and the effect lasted for more than 3 hours. In immunomodulation and tumor research, flavonoids inhibit the infiltration of M2-type macrophages (for instance, quercetin reduces the proportion of such cells in tumor tissues by 30%), regulate the TME, and indirectly assist in tumor suppression, providing support for inflammation and tumor adjuvant therapy [4].

4. The compatibility strategy enhances the clinical application value of *Clematis chinensis*

Clinically, *Clematis clematis* is often combined with heat-clearing and detoxifying TCMs (such as dandelion and *Isatis root*) to form a synergistic model of "combining warmth and clearing" [14].

Professor Zhou used its combination to treat gastric cancer, aiming to achieve the effect of removing blood stasis, detoxifying and eliminating masses. In clinical practice, *Acacia chinensis* was used to treat gastric cancer. The dosage was adjusted according to the syndrome: for the type of

phlegm and blood stasis intermingle, 15g was combined with 12g of *Acacia chinensis* and 10g of *Curcuma zedoaria*, etc. After two months of treatment in 30 cases, the relief rate of epigastric pain was 73.3%, and the effective rate of mass reduction was 46.7%. For patients with stomach heat combined with cancer toxins and blood stasis, 12g was used in combination with 20g of *Hedyotis diffusis* and 6g of *Coptis chinensis*. After treatment, the improvement rate of stomach heat in 25 cases was 80%, and the average quality of life increased by 15 points. For those with obvious obstruction, the weight of the stone was increased to 20g and 15g was punctured. Among the 18 cases, the obstruction was relieved in 12 cases, with an effective rate of 66.7%. Studies have confirmed that 10-20g can enhance the apoptosis rate of gastric cancer cells (20%-35% in vitro), and when combined, it can reduce gastrointestinal irritation [15].

Zhang often used it to promote blood circulation, remove blood stasis and unblock meridians. *Clema chinensis* promotes blood circulation and unblocks meridians. When 15g of *Clema chinensis* is combined with 10g of peach kernels and 8g of safflower to block the epigastric region, the relief rate of epigastric stabbing pain in 32 cases after 4 weeks was 71.9%, and the improvement rate of mucosal ecchymosis was 56.3%. For the combination of phlegm-dampness and dampness, 12g was used in combination with 9g of *Pinellia ternata* and 15g of *Poria cocos*. After two months, the disappearance rate of belching and fullness in 28 cases was 64.3%, and the stability rate of the lesion increased by 18%. The cancer pain significantly increased to 20g combined with 15g of *Corydalis yanhusuo*. Among the 19 cases, the pain score of 14 cases decreased by ≥ 3 points, with an effective rate of 73.7%. Moreover, it reduced blood viscosity by 22% and inhibited the proliferation of gastric cancer cells by 38% to 45% [16]; Professor Zhou uses it for esophageal cancer to improve symptoms such as difficulty in swallowing. For the type of obstruction caused by the interplay of phlegm and qi, 15g was combined with 10g of *Schizothorax* and 9g of *Pinellia ternata*. One month later, the relief rate of dysphagia in 35 cases was 74.3%, and the average obstruction score decreased by 2 points. For those with internal blood stasis, 18g was combined with 15g of *Salvia miltiorrhiza* and 10g of turmeric. Among the 28 cases, the smoothness of swallowing was improved in 20 cases, with an effective rate of 71.4%. When the obstruction was severe, the weight was increased to 20g and a stone was added to puncture 15g. Among the 19 cases, 13 cases could enter the semi-liquid, with an effective rate of 68.4%. Studies have shown that this combination enhances the relaxation of esophageal smooth muscle (25%-30% in vitro) and reduces the dysphagia score of patients with esophageal cancer [17]; Professor Yu Rencun believes that it can alleviate the obstruction symptoms of esophageal cancer. Clinical application emphasizes syndrome differentiation and dosage adjustment: For obstruction caused by the interweaving of phlegm and blood stasis, 15g of *Perilla fruticosa* combined with 12g of *Trichosanthes japonicum* and 10g of *Salvia miltiorrhiza* was used. After 3 weeks, the relief rate of swallowing obstruction in 30 cases was 70%, and 53.3% could consume soft food. For those with obvious qi stagnation, 12g was added with 9g of dried tangerine peel and 10g of August jelly. Among the 26 cases, the obstruction was alleviated in 19 cases, with an effective rate of 73.1%. When the obstruction was severe, the weight was increased to 20g, and the stone penetration was 15g. Among the 22 cases, 14 cases had improved difficulty in eating, with an effective rate of 63.6%. Studies show that 12-20g of *Clematis chinensis* can promote the peristalsis of esophageal smooth muscle (increasing the frequency in vitro by 20%-28%) and assist in relieving obstruction [18]; Wang used it as a prescription to treat uterine fibroids. For fibroids of qi stagnation and blood stasis type, 15g of *Clematis chinensis* was combined with 10g of *Curcuma zedoaria* and 10g of *Turmeric zedoaria*. After 3 months, the effective rate of fibroid shrinkage in 32 cases was 56.2%, and the relief rate of abdominal pain was 75%. For those with phlegm-dampness, 12g was added to 9g of *Pinellia ternata* and 15g of *Poria cocos*. Among the 25 cases, the fibroids shrank in 16

cases, with an effective rate of 64%. When the fibroids were larger than 5cm, they increased to 20g combined with 15g of turtle shells. Among the 18 cases, 10 cases shrank by ≥ 1 cm, with an effective rate of 55.6%. Studies have shown that this combination inhibits fibroid angiogenesis (reducing vascular endothelial factor by 22% to 30% in vitro) and helps to reduce volume [19]; Wu et al., used it in combination with medicine to treat breast fibromas.

Studies have confirmed that this combination strategy can enhance the anti-tumor efficacy of drugs through the mechanisms of clearing heat and detoxifying as well as promoting blood circulation and removing blood stasis, and improve the quality of life and immune function of patients [11, 17].

5. Combined radiotherapy

Clinical studies have shown that the compound Tianxian Capsules, which are composed of the TCM *Clematis auriculata* as the principal ingredient, have demonstrated a significant synergistic effect when combined with radiotherapy in the treatment plan for patients with advanced esophageal cancer. This combined treatment plan can not only significantly improve the long-term survival rate of patients, but also effectively alleviate their clinical symptoms [14, 15]. Specific data shows that the 5-year survival rate of patients in the treatment group treated with Compound Tianxian Capsules combined with radiotherapy was as high as 22.22%, which was significantly better than the 7.4% of the radiotherapy alone group. 0.05. In terms of symptom improvement, the improvement rate of dysphagia symptoms in the combined treatment group reached 85.19%, which was also significantly higher than 62.96% in the radiotherapy alone group, and the statistical difference was statistically significant [20].

6. Conclusion

This study focuses on the efficacy of *Clematis chinensis* in the treatment of OSCC, and summarizes the anti-tumor effects exerted by its core active components, triterpene saponins, flavonoids, and polysaccharides, through multi-target mechanisms. Specifically, it is manifested as activating the apoptotic signaling pathway, blocking the cell cycle, inhibiting invasion and metastasis, and simultaneously regulating the tumor microenvironment. Moreover, the combination and application under the guidance of TCM theory can enhance therapeutic effects and reduce toxicity. The above research achievements have opened up a new path for the integrated treatment of tumors with traditional Chinese and Western medicine, which is in line with the initial goal of this study, that is, to explore the value of TCM in tumor treatment, and provide a reference basis for subsequent related research in dimensions such as multi-target action mechanisms and compatibility applications. However, there are still deficiencies in the precise separation and structural optimization of the active ingredients of *Clematis chinensis* at present, and the sample size and quality of clinical research also need to be improved. Future research should further promote the optimization of active ingredients, combine advanced technologies to analyze molecular networks, carry out more high-quality clinical studies, and explore more optimized drug administration strategies to facilitate the practical application of *Clematis chinensis* in tumor treatment.

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