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Review

Application of Natural Medicinal Plants Active Ingredients in Oral Squamous Cell Carcinoma*

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ABSTRACT Oral squamous cell carcinoma (OSCC) is the most common malignant cancer of the head and neck, with high morbidity and mortality, ranking as the sixth most common cancer in the world. The treatment of OSCC is mainly radiotherapy, chemotherapy and surgery, however, the prognosis of patients is still poor and the recurrence rate is high. This paper reviews the range of effects of natural medicinal plant active ingredients (NMPAIs) on OSCC cancer, including the types of NMPAIs, anti-cancer mechanisms, involved signaling pathways, and clinical trials. The NMPAIs include terpenoids, phenols, flavonoids, glycosides, alkaloids, coumarins, and volatile oils. These active ingredients inhibit proliferation, induce apoptosis and autophagy, inhibit migration and invasion of OSCC cells, and regulate cancer immunity to exert anti-cancer effects. The mechanism involves signaling pathways such as mitogen-activated protein kinase, phosphatidylinositol 3 kinase/protein kinase B, nuclear factor kappa B, miR-22/WNT1/β-catenin and Nrf2/Keap1. Clinically, NMPAIs can inhibit the growth of OSCC, and the combined drug is more effective. Natural medicinal plants are promising candidates for the treatment of OSCC.

KEYWORDS natural medicinal plant, active ingredients, oral squamous cell carcinoma, mechanism, signaling pathway, review

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world, occurring in the mouth, pharynx, hypopharynx, and larynx, with approximately 275,000 cases per year.[1] Oral squamous cell carcinoma (OSCC) is the most common malignant cancer in the head and neck.[2] To date, despite advances in radiotherapy, chemotherapy, and surgery, the prognosis of OSCC patients remains poor, with a low overall survival rate of 50% at 5 years and a high rate of recurrence,[3] a great deal of patients died of metastasis, immunosuppression and relapse.[4] Therefore, it is urgent to discover more sensitive and specific new drugs to improve the early diagnosis and targeted therapy of OSCC. With the development of biochemistry and pharmacology, natural medicine has been proved to be an effective supplement to conventional cancer treatment. As anticancer drugs, natural medicinal plant active ingredients (NMPAIs) have the advantages of safety and small side effects, and have become a hot spot in the cancer clinical treatment. NMPAIs are great valuable in drug development, and about 80%–83% of the approved antineoplastic drugs are natural products or their derivatives.[5]

Lately, plenty of research have focused on the role of NMPAIs in OSCC. This review aims to explore the effects of NMPAIs against OSCC and emphasize the mechanism of their impact on OSCC, with a view to provide a basis for the development of new drugs for OSCC and improve treatment strategies.

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NMPAIs against OSCC

NMPAIs have great advantages in the treatment of oral cancer.[6] The properties and characteristics of various active ingredients are shown in Appendix 1. Some active ingredients and drug sources targets for the treatment of OSCC are presented in Appendix 2. The anti-OSCC NMPAIs mainly include terpenoids, phenols, flavonoids, glycosides, alkaloids, and volatile oils.

Terpenoids

The terpenoids with anti-OSCC effect include betulinic acid,[7] andrographolide,[8] triptolide,[9] pristimerin,[10] arglabin,[11] glaucocalyxin A,[12] ginkgolide[13] and other components. On one hand, terpenoids inhibit the proliferation, migration and invasion, and promote apoptosis of OSCC. For example, betulinic acid,[14,15] pristimerin[10] and glaucocalyxin A[12] inhibited CAL-27, Tca-83, KB, SCC-15 cells proliferation and induced apoptosis; triptolide inhibited the growth of SAS cells and cell xenografts *in vitro* and *in vivo*;[9] arglabin induced apoptosis in SCC-4 cells, arrested cell cycle and inhibited cell metastasis, reduced the tumor volume and growth of transplanted tumors;[11] ginkgolide enhanced apoptosis in Ca9-22 and Ho-1-N-1 cells.[13] On the other hand, terpenoid resists tumor growth by inhibiting cancer stem cells (CSCs), for instance, andrographolide can significantly reduce the tumorigenicity of $ALDH1^{+}CD44^{+}$ OCSCs and restore their radiosensitivity, inhibiting the tumor invasiveness.[8]

Phenols

Phenols with anti-OSCC effects include polydatin,[16] resveratrol,[17] 6-shogaol,[18] magnolol,[19] honokiol,[20] 4-O-methylhonokiol,[21] thyme phenol,[22] carvacrol,[23] curcumin,[24] demethoxy curcumin[25] and carnosic acid,[26] etc. Similar to terpenoids, phenols suppress tumors by interfering with cancer cells and CSCs. The anti-OSCC effect of phenols is mainly to inhibit the migration and invasion of cancer cells, among which polydatin on CAL-27 and Ca9-22 cells,[16] resveratrol on CAL-27, SCC15 and SCC25,[27] YD-9 and YD-38 cells,[28] 6-gingerol on YD-10B and Ca9-22 cells,[18] carvacrol on CAL-27 cells,[23] curcumin on HSC-4 and CA9-22 cells,[24] and carnosic acid on CAL-27 and SCC-9 cells[26] have been shown to inhibit cancer cell migration and invasion. In addition, phenols also inhibited the proliferation and growth of OSCC cells. Polydatin, resveratrol, honokiol, demethoxycurcumin and carnoic acid decreased OSCC cells survival rate and inhibited the proliferation and growth of CAL-27 and Ca9-22,[16,17] HSC-3,[29] HSC-4,[30] OC2 and OCSL,[20] and SCC-9 cells,[25,26] and exerted anticancer activity in OSCC-transplanted mice,[20] and inhibited the tumor growth in CAL-27 and SCC-9 xenografted BALB/c nude mice.[26] Moreover, phenols can also induce autophagy and apoptosis of OSCC cells. Polydatin[16] and honokiol[20] induced autophagy in CAL-27 and Ca9-22, OC2 and OCSL cells, respectively. Resveratrol induced apoptosis in SCC-Ⅶ, SCC-25 and YD-38 cells;[31] the apoptosis of PE/CA-PJ41, CAL-27 and SCC-9 cells was induced by 4-O-methyl honokiol,[21] thymol[22] and carnosic acid,[26] respectively. Similarly, magnolol decreased IL-6 secretion and Stat3 phosphorylation in SAS, OECM1, and GNM stem cells, and inhibited stemness of cancer. [19]

Flavonoids

Flavonoids with anti-OSCC effects included nobiletin,[32] deoxypodophyllotoxin,[33] quercetin,[34] baicalein,[35] jaceosidin,[36] fisetin,[37] psorachromene,[38] and licochalcone A,[39] etc. Nobiletin inhibited cancer occurrence and development, promoted Ca9-22, HSC-3 and TSC-15 cell apoptosis and inhibited cell proliferation,[32] induced cell cycle arrest and suppressed TCA-8113 and CAL-27 cells proliferation.[40] Deoxypodophyllotoxin resisted angiogenesis, significantly reduced cell viability, and induced apoptosis of HSC-2 and HSC-3 cells.[33] Quercetin promoted Tca-8113 and SCC-15 cells apoptosis,[34,41] inhibited the OSC20, SAS, HN22, Tca8113 and SAS cells viability and cell migration.[42,43] Baicalein inhibited cell proliferation *in vivo* and *in vitro*, and blocked cell cycle, induced SCC-25, CAL-27 and HSC-3 cells apoptosis, reduced BALB/c mouse SCC25 cells xenograft tumor volume size.[35] Jaceosidin inhibited HSC-3 and Ca9-22 cells proliferation, aggregation, and induced apoptosis.[36] Fisetin enhanced Ca9-22 cell apoptotic by inhibiting autophagy.[37] Psorachromene induced cell cycle arrest and apoptosis in SAS cells.[38] Licochalcone A inhibited SCC-4 and CAL-27 cell proliferation, migration and invasion.[39]

Glycosides

Anti-OSCC glycosides include ergoside,[44] cordycepin,[45] dioscin,[46] methylprotodioscin,[47] diosgenin,[48] and icariin.[49] Ergoside spawned the apoptosis of HN4 and HN6 cells and inhibited metastasis;[44] cordycepin induced autophagy in HSC-4 cells, inhibited cell growth, impaired the migration and invasion,[45] as well as induced apoptosis in

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OEC-M1 and OC3 cells[50-52] and SAS cells;[52] dioscin significantly induced cell cycle arrest, increased apoptosis and inhibited proliferation in SCC-15 cells;[46] methylprotodiosgenin induced SAS and SCC-9 cells cycle arrest, apoptosis and autophagy;[47] diosgenin reduced PE/CA-PJ15 cells migration, increased cell apoptosis and altered cell cycle;[48] and icariin inhibited the proliferation and induced apoptosis of SCC-9 and CAL-27 cells,[49] reduced the viability and invasion of SCC-9 and SCC-15 cells in a concentration-dependent manner.[53]

Other Active Ingredients

Active substances that have an anticancer effect on OSCC also include alkaloids, coumarin, and volatile oils, among which alkaloids include evodiamine,[54] berberine[55] and paclitaxe.[56] Evodiamine inhibited the HSC-4 cells proliferation, invasion and angiogenesis;[54] paclitaxel inhibited the HSC4 and OSC19 cells growth;[56] berberine down-regulated the oncogenicity of CSCs from SAS cells *in vitro* and attenuated tumor growth in BALB/c mice *in vivo*.[55] Esculetin is the main anti-OSCC coumarin, which plays a role by inhibiting cancer cell growth, inducing cell cycle arrest and promoting cell apoptosis in HN22 and HSC4 cells,[57] SAS cells,[58] HN22 and HSC2 cells.[59] In addition, the volatile oils of natural medicinal plants also have an anti-OSCC effect. *Levisticum officinale* participated in the apoptosis, growth and cell cycle of UMSCC1 cells; the content of monoterpenoids is the most, of which α-Terpinyl acetate containing 48.15%, followed by β-phellandrene 13.16%, β-myrcene 5.07%.[60]

Mechanism of Anti-OSCC Action of NMPAIs

The resistance of the above active ingredients against OSCC is reflected in the intervention of cancer cells and CSCs, inducing apoptosis and autophagy, inhibiting cell proliferation, migration and invasion, and regulating inflammation and tumor immunity (Figure 1 and Appendix 3). Interestingly, these mechanisms are consistent with the above-mentioned situation that a large number of patients with OSCC died of metastasis, immunosuppression and recurrence, and it may be effective to treat patients by intervening in these mechanisms.

Induction of Apoptosis

Apoptosis involves the activation, expression and regulation of a series of genes, and the evasion of apoptosis is an important sign of tumorigenesis. Promoting cancer cell apoptosis is one of the important ways to inhibit cancer development. NMPAIs act on OSCC through down-regulation of anti-apoptotic protein B-lymphocytoma-2 (Bcl-2),[15,18] up-regulation of pro-apoptotic proteins BAX and BAK,[37] tumor suppressor gene expression or inactivation (p53),[15] increasing caspases (caspase-3, -8, -9) expressions[20] and reactive oxygen species (ROS)[11] to maintain the apoptosis of cancer cells, so that cancer cells shrink in size, connection disappears, and the surrounding cells detachment is followed by increased cytoplasmic density, loss of mitochondrial membrane potential,[26] altered permeability, release of cytochrome c into the cytoplasm,[7] nucleoplasmic condensation, fragmentation of the nuclear envelope and nucleoli, and DNA degradation.[32] Noteworthily, the expression

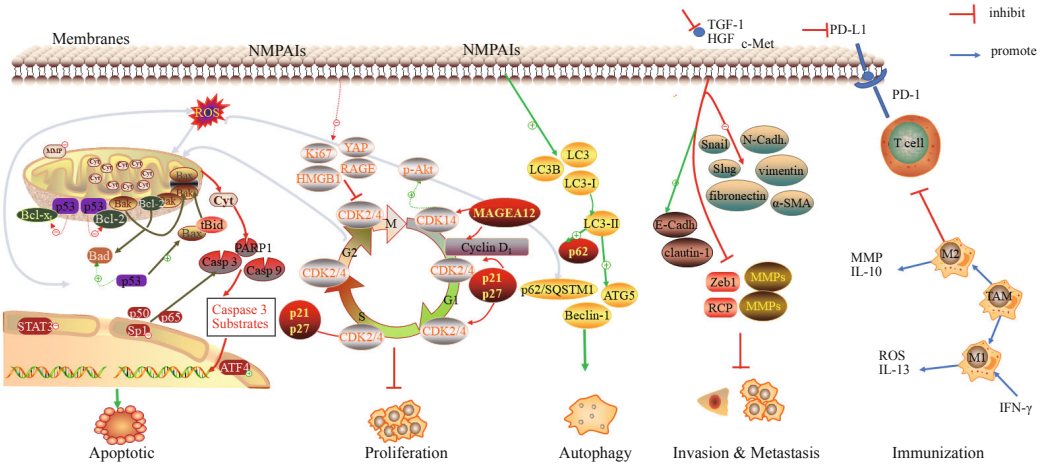

Figure 1. Signaling Mechanisms of Anti-OSCC

Notes: NMPAIs: natural medicinal plant active ingredients; ROS: reactive oxygen species; MMP: matrix metalloproteinase; HMGB1: high-mobility group box 1; N-Cadh: N-cadherin; PD-L1: programmed death-ligand 1; IFN-γ: interferon gamma; TGF: transforming growth factor; HGF: hepatocyte growth factor; OSCC: oral squamous cell carcinoma

regulation of these apoptotic proteins and genes does not function alone, but more of a cascade reaction after activation, which is regulated by various signal transduction pathways.

Regulation of Bcl Family

The Bcl-2 family is an important regulatory factor that mediates the endogenous pathway of apoptosis. It controls the permeability of the mitochondrial outer membrane by regulating the membrane potential, and can be classified into 3 categories: anti-apoptotic proteins, pro-apoptotic proteins, and BH3-only proteins. Anti-apoptotic protein members include Bcl-2, Bcl-XL, Bcl-w, Mcl-1 and BFL-1 (A1); pro-apoptotic protein members include BAX and BAK, BH3-only proteins include Bid, Bim, Bad, Bmf, Bik/Nbk, Blk, Noxa, Bbc3 and DP5, etc.; BH3-only protein increases under stimuli such as cell injury. On the one hand, it can directly activate the pro-apoptotic protein BAX/BAK to form oligomers on the mitochondrial membrane, leading to an increase in the permeability of the outer mitochondrial membrane and eventually apoptosis, alternatively, it binds to anti-apoptotic protein to block the anti-apoptotic effect.[64] Betulinic acid[15] and 6-shogaol[18] could increase the expression of BAX in KB, YD-10B and Ca9-22 cells, reduce the expression of Bcl-2 and the mitochondria oxygen consumption to promote mitochondrial apoptosis, leading to cancer cell apoptosis. Fisetin significantly reduced Bcl-2 and Bcl-XL in Ca9-22 cells, causing accumulation of pro-apoptotic members BAX and BAK.[37] Carnosic acid up-regulated BAX and Bad, downregulated Bcl-2 expression, and induced CAL-27 and SCC-9 cells mitochondrial apoptosis.[26] Quercetin reduced tumor incidence and induced apoptosis through modulation of target genes Bcl-2 and BAX in the DMBA-induced carcigenesis hamster model.[34]

Regulation of Caspase

The caspase family belongs to cysteine proteases, which are the key enzymes that cause apoptosis. Once the signal transduction pathway is activated, caspases are activated, followed by cascade reactions of cascades of caspases.[65] Activated caspase-3 is considered as a biomarker of apoptosis. Poly ADP-ribose polymerase (PARP) is the cleavage substrate of caspase. PARP cleavage is considered to be an important step in apoptosis, also generally regarded as an indicator of caspase-3 activation.[66] Caspase-dependent apoptosis is involved in the anticancer effect of NMPAIs. Betulinic acid[15] and 6-shogaol[18] could increase the activation of caspase-3, -9 in KB, YD-10B and Ca9-22 cell mitochondrial apoptosis; honokiol increased the activation of caspase-3 and induced OC2 and OCSL cell apoptosis.[20] Demethoxycurcumin had better stability and higher water solubility than curcumin after oral administration, it could increase the apoptosis of HSC-3 and SCC-9 cells by inducing the activation of caspase-8/-9/-3.[25] Carnosic acid up-regulated caspase-3/-9 activation levels and induced mitochondrial apoptosis in SCC-9 and CAL-27 cells.[26] Glaucocalyxin A enhanced the cleaved caspase-3 in SCC-25 and CAL-27 cells.[12] Jaceosidin significantly increased the activation of caspase-9/-3 and PARP in a dose-dependent manner, and activated the caspase pathway to induce apoptosis in HSC-3 and Ca9-22 cells.[36] Fisetin activated caspase-3 and PARP, induced Ca9-22 cell apoptosis through the mitochondrial pathway,[37] and esculetin activated caspase-8, and induced SAS cell apoptosis.[58]

Upregulation of Tumor Suppressor Genes

A well-known tumor suppressor gene p53 is regarded as the "guardian of the genome",[67] playing a key role in controlling cell cycle checkpoints and apoptosis by regulating a series of target genes.[68] It was found that betulinic acid increased the activity of p53 reporter gene and p53 binding in Bax promoter, and regulated the expression of p53 in KB cells and implanted tumors.[15] 6-gingerenol regulated apoptosis of YD-10B and Ca9-22 cells induced by p53, Bax, Bcl-2 and cleaved caspase-3.[18]

Regulation of Transcription Factors

Recombinant specificity protein 1 (Sp1) is a ubiquitous transcription factor and acts by binding to the promoters of its target genes, including matrix metalloproteinase (MMP) 3, MMP9, and cyclin D1. Sp1, a negative prognostic survival factor, is overexpressed in many types of cancers, such as breast cancer,[69] gastric cancer,[70] pancreatic cancer, thyroid cancer, liver cancer and gliomas.[71] Sp1 could down-regulate the expression of phosphatase and tensin homolog (PTEN), and by binding to a specific site on the PTEN promoter, betulinic acid increased radiosensitivity and induced Sp1 threonylation to down-regulate Sp1 and up-regulate PTEN expressions in CAL-27 and Tca-83 cells.[7] Baicalein inhibited the expression of Sp1, p65 and p50 by down-regulating the level of related mRNA. The knockout of Sp1 gene also led to the decrease of

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the expression of p65 and p50. Sp1 silencing enhanced the effect of baicalein by inhibiting the SCC-25, CAL-27 and HSC-3 cells growth through Sp1/nuclear factor kappa b (NF-κB)-dependent mechanism.[35] Esculetin inhibited Sp1 and Sp1 regulatory proteins to suppress HN22 and HSC4 proliferation and induce apoptosis.[57]

Signal transducer and activator of transcription 3 (STAT3) is an important transcription factor that is overexpressed in several types of cancer. Inhibition of STAT3 signal has been proved to suppress the development of oral cancer.[72,73] Betulinic acid decreased the phosphorylation level of STAT3 and significantly inhibited STAT3 signal transduction in KB cells.[15]

The transcription factor C/EBP-homologous protein (CHOP) is the core mediator of ER stress-mediated apoptosis, one of the common transcription factors of cation transport regulator-like protein 1 (CHAC1). Activating transcription factor 4 (ATF4) can bind to CHOP to promote CHOP transcription, leading to cell death.[74] Enhanced activation of the ATF4/CHOP axis is a hallmark of maladaptation against ER stress. CHAC1 has critical roles in OSCC development, it is also the downstream target of the ATF4-CHOP axis, which is regulated by ATF4 and CHOP.[75] Glaucocalyxin A could activate the ATF4/CHOP/CHAC1 axis in SCC-25 and CAL-27 cells to induce ER stress-mediated apoptosis.[12]

Increase of ROS

ROS is a group of highly reactive substances that can destroy a variety of molecules or alter a range of signal pathways. The increase of ROS is considered to be an important determinant of apoptosis. Oxidative stress is associated with increased production of ROS, which in turn promotes cancer development and progression.[76] The promotion of ROS generation plays a crucial role in the chemoprevention of various anticancer drugs.[77,78] Betulinic acid significantly increased ROS generation, and inhibited KB cells apoptosis.[15] Arglabin increased the intracellular ROS level of SCC-4 cells by about 200% to induce apoptosis. Mitochondria played an important role in the process of apoptosis.[11] Glaucocalyxin A accumulated excessive ROS to induce mitochondrial apoptosis in SCC-25 and CAL-27 cells;[12] fisetin reduced mitochondrial membrane potential, Bcl-2 and Bcl-xL in Ca9-22 cell, and induced the accumulation of ROS.[37]

Inhibition of Cell Proliferation

Unchecked cell proliferation due to failure of cell cycle arrest is an important hallmark of cancer. In addition, the overexpression and knockdown of related protein factors became the proliferation markers of oral cancer cells. Betulinic acid, honokiol, quercetin, nobiletin, resveratrol and other natural active ingredients can block cell cycle and inhibit/activate tumor proliferation-related proteins in inhibiting cell proliferation.

Cell Cycle Arrest

Cyclin and cyclin-dependent kinases (CDKs) are important molecules in cell cycle regulation. Cyclin is one of the driving forces of cell cycle operation, its expression level can affect the cell cycle, and the change of cell cycle affects cell proliferation. Betulinic acid induced significant G_0/G_1 cell cycle arrest by regulating KB cells cyclin D1, and decreased the number of cells in S phase.[15] Honokiol blocked G_0/G_1 phase in a dose-dependent manner, gradually shortened S phase and G_2/M phase, and inhibited the growth of OC2 and OCSL cells, which was consistent with the downregulation of CDK2 and CDK4 and upregulation of cell cycle inhibitors p21 and p27.[20] Quercetin increased the number of OSC20 and SAS cells in G_2/M phase and decreased the proportion of cells in G_0/G_1 phase.[42] Nobiletin inhibited the proliferation of TCA-8113 and CAL-27 cells and inhibited the aerobic glycolysis of tumor cells by inducing cell cycle arrest in the G_1 phase.[40]

Inhibition/Activation of Tumor Proliferation-Related Proteins

Overexpression of MAGEA12 increased the viability of CAL-27 cells and promoted cell proliferation. Resveratrol decreased the expression of MAGEA12 and phosphorylated(p)-AKT (p-AKT), and inhibited the growth and MAGEA12/AKT signal pathway of CAL-27 cells.[17] Overexpression of metastasis-associated protein 1(MTA1) is associated with the progression of head and neck cancer,[79] and triptolide inhibited the expression of MTA1 in xenograft tissue and SAS cell line in a time- and dose-dependent manner to inhibit the proliferation of OSCC.[9] Ki67 antigen is an immune protein associated with cell division and proliferation in the nucleus. Glaucocalyxin A effectively inhibited the expression of Ki67 in SCC-25 and CAL-27 cells, thus inhibiting cell proliferation.[12] Icariin significantly down-regulated Ki67 in SCC-9 and SCC-15 cells.[53] Ras-associated domain containing protein 1A (RASSF1A)

supports the phosphorylation of MST2 and SAV, both upstream molecules of the yes-associated protein (YAP) tumor suppressor protein Ras.[80] RASSF1A was activated and YAP was phosphorylated by dioscin, YAP overexpression/knockdown decreased/enhanced the inhibitory effect of dioscin on SCC15 cells, respectively; dioscin up-regulated the expression of RASSF1A in SCC15 cells, thus weakening its transcriptional co-activation function and inhibiting cell proliferation.[46] Receptor for advanced glycation end products (RAGE), a receptor of high-mobility group box 1 (HMGB1), is a polyligand member of the immunoglobulin superfamily of cell surface molecules closely related to tumorigenicity. RAGE silencing inhibited HSC-4 cells proliferation and formation of human umbilical vein endothelial cells (HUVEC) tubes. Evodiamine decreased HMBG1 and RAGE to inhibit HSC-4 cells, the tumor size of evodiamine in mouse OSCC xenotransplantation model was consistent with the levels of HMBG1 and RAGE in tumor tissue.[54]

Induction of Autophagy

Another form of cell death is autophagy. Autophagy was formed in Ca9-22 and CAL-27 cells treated with polydatin and increased expression of autophagy-associated proteins ATG5 and LC3.[16] The expression of LC3 and immunofluorescence intensity of LC3 gene in HSC-4 cells treated with cordycepin increased significantly, indicating that cordycepin induced autophagy in HSC-4 cells.[45] Honokiol increased LC3-Ⅱ-induced autophagy in OC2 and OCSL cells, p62 expression increased and autophagic flux continued.[20] Acid vesicular organelles (AVO) is the characteristics of autophagy vacuoles. AVO and LC3B were produced in the cytosol after fisetin treatment in Ca9-22 cells. AVO could induce the formation of autophagy vacuoles, LC3B converted LC3-Ⅰ into LC3-Ⅱ and caused the accumulation of Beclin-1 and ATG5 in Ca9-22 cells in a dose-dependent manner, while the expression of p62/SQSTM1 decreased.[37]

Inhibition of Cancer Cell Migration and Invasion

Cancer cell migration and invasion are effective ways for cancer development via inhibiting epithelial-mesenchymal transition (EMT), regulating MMP and microrRNAs to weaken or even inhibit the migration and invasion of OSCC.

Regulation of EMT

EMT is the basis of malignant epithelial tumorigenesis, it can promote the metastasis and invasion of tumor cells, thus blocking the process of EMT is of great significance for anti-tumor. NMPAIs can inhibit the invasion and metastasis of OSCC by increasing the expression of EMT-related factor E-cadherin (epithelial marker), reducing N-cadherin (interstitial marker) and Snail (marker of mesenchymal phenotype), and blocking EMT-induced transcription factor (EMT-TF) zinc finger E-box-binding protein1 (Zeb1). Polydatin increased the expression of E-cadherin and reduced the expression of N-cadherin and zinc finger transcription factor Slug and Snail protein in CAL-27 and Ca9-22 cells.[16] Cordycepin and 6-shogaol up-regulated E-cadherin and down-regulated N-cadherin in YD-10B, Ca9-22 and SAS cells.[18,52] Quercetin increased the expression of E-cadherin and clautin-1 while decreased the expression of mesenchymal markers such as fibronectin, vimentin and alpha-smooth muscle actin (α-SMA) in OSC20, SAS and HN22 cells in a dose-dependent manner.[42]

Zeb1 was recently identified as an indicator of recurrent OSCC, and blockade of Zeb1 resulted in regression of OSCC metastases.[81] Rab coupling protein (RCP) stabilizes β1 integrin through circulating nucleosomes, preventing it from lysosomal degradation and activation of downstream signal cascades of epidermal growth factor receptor (EGFR), ultimately resulting in increased cancer invasion and metastasis.[82,83] Zeb1 is located downstream of β1 integrin/EGFR signaling axis. RCP and Zeb1 increase cancer invasion-related proteolytic enzyme membrane type 1 matrix metalloproteinase (MT1-MMP),[84,85] and RCP can positively regulate the invasiveness of OSCC through the expression of Zeb1 and MT1-MMP. Resveratrol effectively reduced the invasion of YD-9 and YD-38 cells induced by RCP through down-regulating the expression of β1 integrin and Zeb1.[28] Furthermore, hepatocyte growth factor (HGF) signaling pathway induced EMT through downstream activation of HGF receptor c-Met and survival-promoting extracellular regulated protein kinases (ERK) pathway. Curcumin inhibited HSC4 and Ca9-22 cells invasion and migration by down-regulating the expression of phosphorylated c-Met (ERK) and E-cadherin, increasing vimentin, and reversing HGF-induced EMT.[24]

Inhibition of MMP

MMPs are important proteolytic enzyme for cancer migration and invasion. MMP2 and MMP9 are

highly elevated and associated with accelerated tumor progression, decreasing transforming growth factor (TGF) and activation of MMP2 and MMP9, inhibited the expression of EMT inducers and MMPs, as well as metastasis and invasion of cancer cells in OSCC.[42] Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that helps regulate cell adhesion, metastasis and survival, as well as the expression and activity of MMP2 and MMP9. AKT/protein kinase B is the core member of phosphatidylinositol 3 kinase (PI3K) and one of the downstream effectors of FAK. PI3K/AKT signal promotes metastasis by up-regulating MMP2 and MMP9.[45] Cordycepin significantly decreased the ratios of p-FAK/FAK and p-AKT/AKT, down-regulated MMP2 and MMP9 mRNA expressions, inhibited the HSC-4 cell migration and invasion.[45] Curcumin inhibited ERK/MAP kinase and NF-κB activation, reduced the activity of MMP-2/9 and inhibited cell motility in YD-10B cells.[86]

Regulation of MicroRNAs

MicroRNAs (miRNAs), a small non-coding RNA, as a cancer oncogene suppressor, is involved in important biological processes of cancer apoptosis and invasion.[87] Quercetin up-regulated miR-1254 and down-regulated CD36, and the overexpression of miR-1254 also down-regulated CD36, significantly inhibiting the invasion of CAL-27 cells in a dose-dependent manner;[88] it also promoted the overexpression of miR-22 and inhibited the expression of WNT1 and β-catenin in Tca8113 and SAS cells.[43] Berberine suppressed the expression of miR-21 and reduced the properties of OSCC CSCs, including migration and invasive ability.[55] What's more, high BMI1 expression is often associated with advanced stage, aggressive clinicopathological behavior, stem cell-like properties, drug resistance and poor prognosis for HNSCC,[89] and study suggested that the interaction between miR-and BMI1 determines the life form of OSCC CSCs.[90] Experiments indicated that andrographolide treatment increased the expression of miR-218, leading to the downregulation of BMI1 and inhibited the tumor invasiveness of $ALDH1^{+}CD44^{+}$ OSCC CSCs *in vitro*. miR-218 knockout enhanced tumor stemness, while silencing counteracting this effect. Tumor growth in xenograft-bearing mice was reduced after andrographolide treatment by activating the miR-218/BMI1 axis *in vivo*.[8]

Regulation of Inflammation and Immunization

A striking feature of cancer cells is that they can evade destruction of the immune system by exploiting several mechanisms. The infiltration of tumor-associated macrophages (TAMs) was also significantly correlated with the high expression of programmed death-ligand 1 (PD-L1) in tumors.[91] PD-L1 has inhibitory function in persistent antigen stimulation environment and acts as an immune checkpoint protein to negatively regulate activated T cells.[92,93] Interferon gamma (IFN-γ) is a multifunctional cytokine produced in tumor microenvironment (TME) and plays a key role in inflammatory response. IFN-γ can promote anti-tumor immune response, and serum IFN-γ levels are higher in patients with head and neck cancer.[94] Kuo, et al[95] found that triptolide inhibited PD-L1 expression in SAS cells and tumor xenotransplantation (PDTX) model, inhibited tumor growth, and expressed in OSCC in IFN-γ-regulated microenvironment. Analysis of clinical research reveals that the inflammatory factors IL-4, IL-13, IL-6 and TNF-α have a very significant contribution to the occurrence of OSCC. High expression of IL-4 is associated with increased recurrence, while the high expression of IL-13 is inversely related to the recurrence of OSCC patients.[96] Peng, et al[19] found that magnolol reduced IL-6 secretion and Stat3 phosphorylation, inhibited stemness of cancer through IL-6/Stat3 signal pathway in SAS, OECM1 and GNM CSCs,[19] and its derivative 2-O-methylmagnolol had stronger inhibitory effect on IL-6/STAT3 signal transduction in SAS cells.[97] Carvacrol inhibited the expression of Keap1/Nrf2/HO-1, EMT-related proteins and NALP3 inflammasome in CAL-27 cells, suppressed proliferation and migration and improved the inflammation.[23]

Anti-OSCC Related Signaling Pathways

The anti-tumor signal pathways of NMPAIs mainly include mitogen-activated protein kinase (MAPK) signal pathway, phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT) pathway, NF-κB signal pathway, miR-22/WNT1/β-catenin signal pathway and Nrf2/Keap1 pathway. Interestingly, these signal pathways may act simultaneously.

The 3 major subfamilies of MAPK kinases include extracellular signal-regulated kinases (ERKs), c-jun N-terminal kinases (JNKs), and p38MAPK kinases. Methylprotodioscin induced SAS and SCC-9 cells apoptosis and autophagy by modulation of p38 MAPK and JNK1/2 pathways.[47] Pristimerin mediated CAL-27 and SCC-15 cells apoptosis by

activating endoplasmic reticulum stress/JNK axis;[10] Deoxypodophyllotoxin inhibited the progression in HSC2 and HSC3 cells by regulating PI3K/AKT and p38 MAPK signal pathway.[33] Jaceosidin induced HSC-3 and Ca9-22 cells apoptosis and inhibited proliferation by AKT pathway.[36] Arglabin down-regulated mammalian target of rapamycin (mTOR) and p-mTOR as well as PI3K and AKT proteins, and induced anticancer and apoptosis effects in SCC-4 cells through mTOR/PI3K/AKT signaling pathway.[11] Esculetin induced apoptosis in HN22 and HSC2 to inhibit cell viability, which is associated with significant inhibition of EGFR/PI3K/AKT signaling pathway.[59] Quercetin inhibited the phosphorylation of AKT and I κ B kinase-β (IKK β), inhibited the expression of NF-κB and x-linked inhibitor of apoptosis protein (xIAP), and promoted apoptosis of cell lines Tca-8113 and SCC-15.[41] Icariin down-regulated toll-like receptor 4 (TLR4)/NF-κB signal pathway and reduced the growth and invasion capacity of SCC-9 and SCC-15 cell,[53] otherwise, icariin decreased the expressions of p-p65, p-PI3K and p-AKT in SCC9 and CAL-27 cells, inhibited proliferation and induced apoptosis by inhibiting NF-κB and PI3K/AKT signaling pathway.[49] Quercetin regulated miR-22 to exert its anti-tumor function in Tca8113 and SAS cells by combining miR-22 downstream pathway WNT1/β-catenin.[43] Carvacrol inhibited the activation of Keap1/Nrf2/HO-1 in CAL-27 cells and improved inflammation of OSCC by Nrf2/Keap1 signaling pathway.[23]

Clinical Research on NMPAIs against OSCC

Regarding clinical data, a few numbers of studies on NMPAIs against OSCC have been conducted. In a study, the alkaloids teniposide extracted from *Cephalotaxus fortune* Hook. and cisplatin were administered intravenously to patients with OSCC, it was found that the growth inhibition rates of teniposide and cisplatin on OSCC cells were 63.34% and 24.08%, respectively, and teniposide showed a good chemotherapeutic effect.[98] In another study conducted by Knobloch, et al,[99] the oral cancer tissues from OSCC patients treated with freeze-dried black raspberry powder were biopsied. It was found that the expression of molecular biomarkers promoting survival genes (Aurora kinase A, baculoviral IAP repeat containing 5, EGFR) and pro-inflammatory genes (nuclear factor kappa B subunit 1, prostaglandin-endoperoxide synthase 2) decreased significantly, and the bioactive components cyanidin-3-rutinoside and cyanidin-3-xylosylrutinoside were detected in the tissue. Additionally, the extract of *Radix Astragali* 12 g, *Fructus Lycii* (F. lycii) 9 g and Jujube 9 g combined with levamisole was used in patients with OSCC. The results revealed the serum squamous cell carcinoma antigen level of patients after treatment was significantly lower than pre-treatment and the combination of drugs was better.[100]

Noteworthily, NMPAIs had been used in combination with chenmotherapy for OSCC patients. Fourteen patients with technically unresectable OSCC were healed by paclitaxel-carboplatin combined with oral metronomic chemotherapy, 9 (65%) patients showed partial response and none of the patients had tumour progression. They were deemed resectable after neoadjuvant chemotherapy, and overall survival at 15 months was 63.5%, indicating that the combined technology is a well-tolerated and less resource intensive NACT regimen, which leads to favourable resection rate and survival.[101]

Summary and Prospect

NMPAIs have gained much consideration as potential anti-OSCC drugs in the past decades because of their different reactions and safer characteristics than chemotherapeutic drugs. However, due to the multiplicity and variability of cancer, the challenge of targeting specific molecules or signal pathways has been proposed. NMPAIs have been found to be effective and shown a synergistic effect with various other ingredients and chemotherapeutic drugs. In the study of OSCC *in vivo* and *in vitro*, the active components of natural medicinal plants have the potential to target a variety of events to hinder tumor progression, induce apoptosis and autophagy, prevent migration and invasion, and regulate immunity. In the clinical treatment of OSCC, the application of natural medicinal plants is not sufficient. Although triptolide could inhibited PD-L1[95] and down-regulate DcR3[9] to inhibit oral cancer xenografts, there are still a limited number of studies that can be used to verify its effects *in vivo* and *in vitro*, which will need to be further studied.

In addition to the above mechanisms, we also have an important discovery. Oral microflora is the second largest community in human body which plays a key role in the occurrence and development of OSCC, including, but not limited to, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella*, *Streptococcus*, *Candida albicans* and viruses.[102] Many reviews state clearly the oral microecology imbalance and

the synergistic effect of oral microbial community can promote the occurrence, progress and transfer of OSCC.[103,104] A meta-analysis implied that clostridium and *Prevosiella* induced pro-inflammatory microenvironment, *Streptococcus acidogenesis* decreased the pH value of OSCC, *Neisseria* increased the sensitivity of irritants to damage mucosa, and PG inhibited macrophages from attacking tumor cells through membrane component molecules to induce OSCC. Viral oncogenes are continuously expressed during the incubation period, in which herpes simplex virus-1 induces the expression of stress or heat shock proteins. By inducing chromosome rearrangement, cells are directly transformed into malignant phenotypes through mutagenic characteristics, which increases the risk of OSCC by 18.8 times.[105] Therefore, maintaining oral microecological balance by regulating oral microflora is one of the mechanisms for the inhibition of OSCC. Massive studies have shown that NMPAIs can maintain oral microecological balance. Curcumin and magnolol can inhibit PG activity and change oral microecology.[106-109] Magnolol can effectively reduce the activity of *Candida albicans*,[110] Curcumin could significantly reduce the activity of *Streptococcus mutans*,[111] and cinnamon essential oil has a good antibacterial activity against *Streptococcus mutans* and *Candida albicans*.[112] Therefore, we speculate that NMPAIs can also inhibit OSCC by regulating the balance of oral microecology, which needs to be verified by further experiments *in vivo*, and it will be important for studying the anti-OSCC mechanism of NMPAIs.

In conclusion, this review provides a certain basis for the clinical research of natural medicinal plants. With continued research on natural medicines and their active ingredients, new mechanisms and target molecules are emerging that will take chemoprevention in cancer treatment to a new level. Nevertheless, there are novel approaches to increase the release and bioavailability of NMPAIs, such as nanoentrapped[113] and microencapsulated[114] forms. More strategies need to be developed to optimize delivery and evaluate pharmacokinetics to improves its healing potential.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Author Contributions

Ren QL and Wang Q designed the review and wrote the initial draft. Ren QL, Zhu Y, Tian T and Li XL drafted the article or revised it critically for important intellectual content. Ren QL, Li XL, Wang M, Wang Q and Liu JG participated in final approval of the version to be submitted. Ren QL, Hu H, Li SH and Shi RY collected the data. All authors were involved in reviewing and editing of the manuscript and approved the final version for publication.

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