Accumulating evidence from epidemiological, pharmacological and case control studies has shown that dihydroartemisinin (DHA), one of first-line antimalarial drugs recommended by World Health Organization (WHO), possesses antineoplastic activity and selective cytotoxicity to a variety of human cancer cell model systems. Although some antitumor mechanisms of DHA have been confirmed, the findings from the previous studies are insufficient to fully reveal the whole picture of tumor suppression by DHA. In a recent investigation, we demonstrated that DHA exhibits antitumor properties against a variety of human HNSCC cells both in vitro and in vivo. The underlying mechanism involves selective inhibition of Jak2/STAT3 signaling. By specific inhibition of STAT3 activation, DHA exerted the chemotherapeutic efficacy, including reduction in cell viability and migratory capability, along with induction of G1 phase cell cycle arrest and apoptosis in head and neck squamous cell carcinoma (HNSCC) cells. All of data suggested that DHA may be a potent inhibitor of STAT3 that can be potentially used to treat patients with HNSCC, as well as other human cancers harboring STAT3 activation.

**Keywords:** HNSCC; head and neck squamous cell carcinoma; DHA; dihydroartemisinin; STAT3; signal transducer and activator of transcription3; inhibitor

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Head and neck squamous cell carcinoma (HNSCC) accounts for more than 90% of head and neck cancers and is the sixth most common malignancy, which causes about 650,000 new cases and 350,000 deaths each year worldwide [1]. Tobacco, alcohol and human papillomavirus (HPV) have been recognized as three important risk factors in HNSCC. The median age of the patients was about 60, with a large male-to-female predominance. According to the authoritative statistical data from China [2], HNSCC including lip, oral cavity, pharynx, nasopharynx, and larynx cancers, without trachea malignancies, accounts for 3.15% of all malignancies arising in the body. Despite that surgery, chemotherapy and radiotherapy, the three major methods currently applied in the treatment of HNSCC, have partially improved clinical outcomes, the overall 5-year survival rate remains embarrassing, approximately 40-50% [3]. The grim outlook in the management of HNSCC poses a great difficulty in devising new targeting drugs, identifying therapeutic targets,
and choosing treatment modalities/strategies to improve the survival and outcome of HNSCC patients.

Signal transducer and activator of transcription 3 (STAT3) protein is one of the STATs family members that comprise seven transcription factors, and has been found to be functioning in regulation of transcription and expression of some important genes responsible for biological events, such as embryonic development, cell survival and death, innate and adaptive immunity and organogenesis in human organisms. Inactive STAT3 monomer is a cytoplasmic protein that can be phosphorylated and activated by cytokines, growth factors and oncoproteins such as Src and Abl [4]. Once phosphorylated, STAT3 becomes homodimerized and/or heterodimerized with STAT1 or STAT5 through reciprocal SH2 domain/phosphotyrosine interactions. Consequently, dimerized STAT3 translocates to the nucleus and bind to promoter regions of specific DNA, and regulates expression of downstream genes that are associated with cell survival and proliferation [5], cell cycle [6], apoptosis [7] and angiogenesis [8].

Normally, the STAT3 activation ceases within a short time for modulation of different negative regulators or dephosphorylation, and the monomer relocates to cytoplasm, waiting for “next loop”. However, persistent phosphorylation of STAT3 has been found in a dozen types of human cancers including head and neck cancers [9], hematologic malignancies [10], pancreatic cancers [11], hepatocellular carcinomas [12], and breast [13] and lung cancers [14]. A number of studies have shown that STAT3 activation plays key roles in the process of pathogenesis and progression of cancers, such as initiation of malignant transformation [15], invasion and metastasis [16], immune evasion and suppression [17], as well as therapeutic resistance to chemoradiation and molecular targeted therapies [18]. Therefore, STAT3 signaling pathway has been considered as an ideal therapeutic target for human cancer treatment [15, 19], and developing agents that can effectively block STAT3 activation may serve as one of the most promising strategies for cancer treatment. To this end, several agents have been identified as effective inhibitor of STAT3 activation [20-22]; however, very few of them have entered clinical trial and passed FDA certification for ambiguous adverse effects and unavoidable major toxicities to the host. Because STAT3 activation is required for growth of HNSCC cells [23], and persistent STAT3 activation is found in more than 95% in HNSCC [24], STAT3 has been proposed as a main therapeutic target for developing therapeutic strategies of HNSCC [15, 25].

The sesquiterpene lactone dihydroartemisinin (DHA) is one of the most effective antimalarial drugs recommended by WHO in combating P. falciparum malaria. As a semisynthetic derivative of artemisinin (also known as Qinghaosu), DHA has also been used to treat malaria for decades. For saving millions of lives from malaria-affected populations, Youyou Tu, a Chinese scientist who is best known for identifying artemisinin and DHA, was awarded the 2015 Nobel Prize in Physiology or Medicine. Apart from antimalarial and anti-schistosomiasis [26] activities, DHA has been shown to exhibit inhibitory effects against erythematous lupus [27], inflammatory diseases [28], bacteria [29] and viruses [30]. However, another conspicuous property of DHA resides on its potent antitumor activities against a variety of human cancer cells, such as lung carcinoma [31], breast cancer [32], hepatocellular carcinoma [33], osteosarcoma [34], acute myeloid leukemia [35] and cervical cancer [36], etc.

We believe that DHA has three dominant advantages over other chemotherapeutic agents: (I) well-understood clinical pharmacokinetics and pharmacodynamics based on its long-term and frontline clinical applications for antimalarial purposes; (II) firmly established large-scale production of DHA, which is beneficial to decrease the cost of chemotherapy; (III) potent antitumor efficacies, negligible adverse effects and easy solubility in water, encouraging its practical uses in cancer treatment. The anti-cancer effects of DHA have been proposed to be mediated through generating reactive oxygen species (ROS) and NOXA [35, 37], reducing PKCα/Raf/ERK and JNK activities [38], and decreasing NF-κB DNA binding [39] and MEK/ERK signaling pathway [40]. However, all of before-mentioned molecular understandings referring to the pan-pathway inhibitory effects on cancer cells are insufficient to explain the peculiar part of tumor inhibition borne by DHA, which deserves further investigation.

Although previous studies confirmed that DHA exerted a striking inhibitory effect on some other cancer cells, no available data about the proliferation inhibition effects of DHA on HNSCC cells have been described so far. In our recent study, we validated the therapeutic potency of DHA toward HNSCC cells both in vitro and in vivo. The growth-inhibitory effects of the compound were associated with a marked G1 phase cell cycle arrest, inhibition of proliferation and induction of apoptosis through selective inhibition of Jak2/STAT3 signaling pathway and subsequent downregulation of downstream target gene proteins. The underlying mechanism involves that DHA decreases the levels of phosphotyrosine indispensable for activation of STAT3 in HNSCC cells. Therefore, we confirmed for the first time that DHA is a potent inhibitor of STAT3 activation that can be potentially used to treat patients with HNSCC and/or to overcome therapeutic resistance for the purpose of improving the outcomes of these patients.
STAT3 molecule has two phosphorylation sites, Tyr705 and Ser727, both of which are activated by two different phosphatase and different routes with distinct functions. Phosphorylation at Tyr705 site is the essential event for STAT3 dimerization, nuclear translocation and DNA binding, while phosphorylation at Ser727 site can be evoked by members of the mitogen-activated protein kinases (MAPK) and c-Jun N-terminal kinase families [441], of which functions remain controversial because the serine phosphorylation has been reported to have both down- and upregulation of STAT3’s transcriptional activities [42, 43]. In our study, we did parallel experiments using antibodies against two phosphorylation sites of STAT3, p-STAT3 (Tyr705) and p-STAT3 (Ser727), and happened to find that DHA only decreased Tyr705 phosphorylation without altering the status of Ser727 phosphorylation in the three HNSCC cell lines (Fadu, Cal-27 and Hep-2). Therefore, inhibition of STAT3 activation by DHA is Tyr705 specific.

Using transient transfection (with DN-Jak2, DN-EGFR, DN-SRC and CA-STAT3 constructs) and IL-6/hypoxia stress stimulation, we made it clear that inactivation of STAT3 by DHA depended on Jak2 kinase activity rather than on inhibition of EGFR tyrosine and SRC family kinases in HNSCC cells. The findings are consistent with Hedvat's conclusion that Jak family kinases are central mediators in modulating STAT3 activation in solid tumor cell lines [444]. Notably, overexpression of constitutively activated STAT3 (CA-STAT3) attenuated DHA-induced HNSCC cell cycle arrest and apoptosis. Furthermore, we also showed that DHA administration did not affect MAPK/RAS and PI3K/Akt signaling pathway, the former of which can activate STAT3 and the latter of which is overactive and frequently seen in human HNSCC [145, 46]. Similarly, compared with AZD1480 and AG490, two specific inhibitors of Jak2, DHA possessed the same activity of suppression of p-Jak2 and p-STAT3 in HNSCC cells. These findings validated DHA as a novel and putative STAT3 inhibitor that selectively inactivates Jak2/STAT3 signaling, and confirmed that inactivation of Jak2 and STAT3 phosphorylation is critical in DHA-mediated proliferation inhibition in HNSCC cells.

We have previously shown that interruption of persistent STAT3 activation by shRNA leads to proliferation inhibition of laryngeal carcinoma cells both in vitro and in vivo [47, 48]. Therefore, we went on to investigate the efficacy and toxicity of DHA in vivo. Quite expectedly, we found that targeted blockade of STAT3 using DHA could substantially suppress the growth of the xenograft HNSCC tumors, without affecting the general conditions of the tumor-bearing animals. In addition, marked reduction of Jak2 phosphorylation and STAT3 activation was observed in xenograft tumors in DHA intervention group. These observations further support the conclusion that tumor inhibition effects seen in HNSCC cells upon treatment with DHA are associated with selective prevention of Jak2/STAT3 signaling pathway. It is for the first time we defined DHA as a novel and putative inhibitor of STAT3 activation, and discovered a fairly new mechanism accounting for proliferation and growth inhibition effects induced by DHA in HNSCC cells both in vitro and in vivo. In view of the fact that DHA shares strong antitumor and chemosensitization activities in different human HNSCC cells, it is absolutely necessary to conduct further and in-depth clinical investigations to explore the plausibility and efficacy of using DHA as a STAT3 inhibitor in the treatment of HNSCC patients.

In conclusion, activation of STAT3 is associated with proliferation, progression and therapeutic resistance of various human cancers. Given our preliminary success in defining DHA as a new STAT3 inhibitor, it is not at all unreasonable to anticipate that DHA can be applied clinically to block STAT3 activation in the treatment of assorted cancers. Therefore, the newly defined STAT3 inhibitor DHA can be potentially used for the purposes of chemoprevention, direct tumor-killing and therapeutic sensitization in the treatment of different malignancies harboring STAT3 phosphorylation. However, the strategies for application of DHA in each potential clinical use must be carefully investigated and has to be individualized among different patients and various cancer types.

Conflicting interests

The authors have declared that no conflict of interests exist.

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