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A folic acid-decorated nanoparticles loaded JQ1 for oral squamous cell carcinoma therapy

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is known as one of the most malignant tumors with high recurrence and fatality rate. The poor tumor-targeting ability of traditional chemotherapeutic drugs has been a grand challenge for anti-OSCC therapy. Beyond that, a large quantity of tumor associated macrophages in OSCC tissues further diminish the anti-tumor effects of these drugs. Therefore, we produced a therapeutic nano drug delivery system (FA-PEG-PLA-JQ1) through encapsulating JQ1 [a small-molecule inhibitor of bromodomain containing protein 4 (BRD4)] into the folic acid (FA)-modified nanoparticle (PEG-PLA), which could prolong the half-life of JQ1 and target the tumor tissues. And then, JQ1 released from this nanoparticle could prevent OSCC growth inducing tumor cell apoptosis, inhibiting tumor angiogenesis and the polarization of M2 type macrophages. In conclusion, our date demonstrated the therapeutic benefits of FA-PEG-PLA-JQ1 against OSCC *in vivo* or *in vitro*, which could be a novel treatment strategy for OSCC in coming days.

Oral cancer, originating from the tongue, buccal mucosa, floor of the mouth, gingival and other parts in the oral cavity, is one of the most common malignant tumors in head and neck region, with more than 350,000 new cases and about 170,000 deaths in 2018 [1,2]. And oral squamous cell carcinoma (OSCC) accounts for more than 95% of oral cancer, which has a high rate of local recurrence and distant metastasis [3]. Surgical resection usually caused the permanent impairment of related function due to the special anatomical position of the oral cavity [4–6]. In recent years, chemotherapy has become the mainstay of treatment for patients with advanced OSCC. However, traditional chemotherapeutic drugs have poor aqueous solubility and stability, drug-related side effects, which hinders its further clinical application [7]. Furthermore, OSCC can promote cancer invasion and progression by inducing the production of M2 polarized macrophages, which leads to drug resistance and blocks antitumor immunity [8]. Thus, there is an urgent need to find more effective drug delivery systems and new therapeutic targets for OSCC patients.

Recently, aberrant epigenetic modifications have been commonly observed in many types of cancer, which could allow cancer cells override growth control and change the tumor microenvironment [9,10]. Histone acetylation is one of the important epigenetic modifications and its epigenetic reader protein is bromodomain containing protein 4 (BRD4) [11]. BRD4, the member of the bromodomain and extra-terminal (BET) protein family, is generally known for its key role in regulating gene transcription for many physiological conditions or diseases, such as cell apoptosis, inflammation and fibrosis [12]. Meanwhile, BRD4 is closely associated with malignancies, which is upregulated in many tumors and involved in tumor proliferation process [13–17]. Given the prominent role of BRD4 in numerous cancers, many BRD4 targeted small molecular compounds have been designed [18]. JQ1 is a high-affinity small molecular inhibitor of BRD4 which can selectively remove BET epigenetic proteins from the acetylated lysine residues, restrain oncogene expression, and eventually inhibit tumor growth

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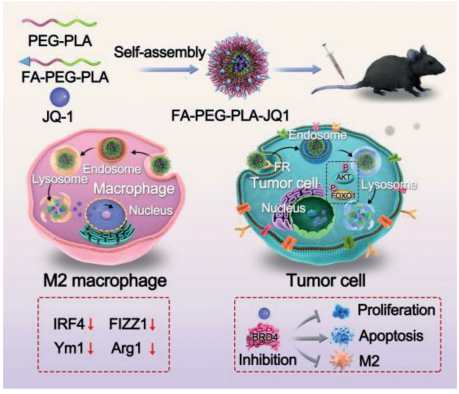
[18]. Numerous studies have demonstrated that JQ1 has potential efficacy for many cancers including triple negative breast cancer, pancreatic cancer, gastric cancer and cervical cancer [19–23]. JQ1 also have shown benefits in retina inflammation and degeneration, acute colon injury, neuroinflammation as well as diabetic cardiomyopathy. Nonetheless, short plasma half-life, poor aqueous solubility of JQ1 greatly limits its further clinical application [24]. Thus, it is urgent to product a new delivery platform to improve the biological availability of JQ1.

Over the past years, nano-drug delivery system has played more and more important role in drug delivery and cancer therapy for improving solubility and enhancing the accumulation of drugs in the tumor site through enhanced permeability and retention (EPR) [25–28]. As a perfect amphiphilic polymer micelle, poly(ethylene glycol)-polylactide (PEG-PLA) polymeric micelles has excellent properties of biocompatibility and biodegradability [29]. For one thing, PEG can serve as a hydrophilic shell to prolong plasma half time through reducing some nonspecific attachment to blood components. For another, PLA is biocompatible and biodegradable which makes it serve as a hydrophobic core to load anticancer drugs [30]. Apart from that, targeting ligands binding to the surface of the nanoparticles could endow nanoparticles tumor-targeting and anti-multidrug-resistant capabilities. As one of the most widely studied cancer targeting ligands, folic acid (FA) could bind to the membrane-bound folate receptor (FR) specially [31,32]. Although its expression is very conservative in normal tissues, FR is usually highly expressed in multiple malignant tumors including ovary, colon, lung, head and neck, and kidney cancers [33]. It is suggested that the FR is significant expressed in more than 50% patients with OSCC [34]. What is more, in the squamous cell carcinomas of the head and neck, patients with highly expressed FR are often more likely to develop lymph node metastases and poorer clinical outcomes than those patients without FR expression [35]. These findings provide the feasibility for FA-mediated nanotherapeutics in OSCC.

In our study, we employed BRD4 as a therapeutic target for OSCC and constructed a tumor-targeting nanoparticle (FA-PEG-PLA-JQ1) to treat OSCC. Subsequently, we investigated the therapeutic efficiency and action mechanisms of this nanoparticle on OSCC at the cellular level through affecting proliferation, apoptosis, and M2 macrophages polarization (Scheme 1). Finally, we established SCC7 tumor-bearing mouse model to study anti-OSCC activity and biosafety of this nanoparticle. The FA-PEG-PLA-JQ1 nanoparticles constructed have good water solubility and small size, which facilitates its passive diffusion through the tumor endothelial junction. In addition, the targeting property of the nanoparticles is greatly improved with the folate conjugation. Compared with other nanoparticles, the nanocomposites have low surface potential, and



Scheme 1. Schematic representation of the formation of FA-PEG-PLA-JQ1.

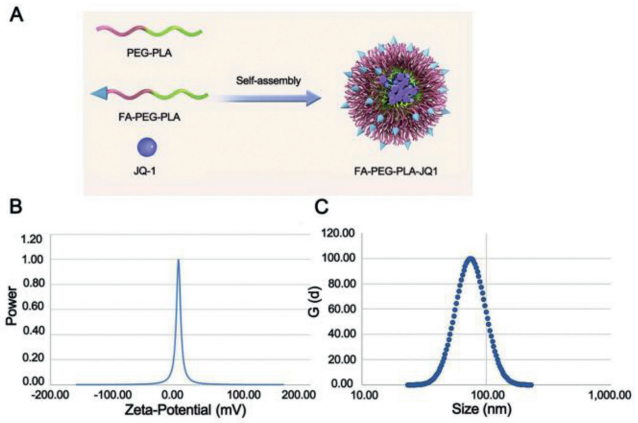


Fig. 1. Preparation and characterization of FA-PEG-PLA-JQ1. (A) Schematic representation of the formation of FA-PEG-PLA-JQ1. (B) Zeta potential of FA-PEG-PLA-JQ1. (C) Size distribution of FA-PEG-PLA-JQ1.

the nanocomposites are stable in blood circulation and slow in degradation, so that the JQ1 can be continuously released, and effectively accumulated in tumors. Our results showed FA-PEG-PLA-JQ1 nanoparticles exhibited good anti-tumor efficiency for OSCC and did not cause obvious toxicity to vital organs, which proved to be a desirable therapeutic drug. We hope that this study can provide a potential strategy for the treatment of OSCC and a basis for clinical drug development.

FA-PEG-PLA-JQ1 was successfully synthesized as shown in Fig. 1A. The zeta-potential and size of FA-PEG-PLA-JQ1 was about −1.33 mV and 38.2 nm (Figs. 1B and C). The drug loading and encapsulation efficiency of FA-PEG-PLA-JQ1 is 5% and 97%, respectively.

We performed MTT assay to explore the viability of OSCC cells (SCC7 cells and HSC2 cells) after treated with Free-JQ1, PEG-PLA-JQ1 and FA-PEG-PLA-JQ1 for 24 and 48 h. As shown in Figs. 2A and B, the activity of OSCC cells decreased with increasing drug concentration and decreased with increasing drug treatment time. When the concentration of JQ1 was 30 μg/mL, the relative viability of SCC7 cells and HSC2 cells in FA-PEG-PLA-JQ1 group at 24 h was about 20% and 40%, respectively. When the concentration of JQ1 was only 0.9 μg/mL, the relative viability of SCC7 cells at 48 h was 50%. When the concentration of JQ1 was 30 μg/mL, the relative viability of SCC7 cells and HSC2 cells in FA-PEG-PLA-JQ1 group at 48 h was about 10% and 20%, respectively, which seemed to prove that SCC7 cells are more sensitive to JQ1 treatment.

Although the effectiveness of Free-JQ1 is not very different from that of PEG-PLA-JQ1. This may be due to the passive diffusion mechanism of Free-JQ1 which allows the drug to enter the cancer cell more quickly in the early stage, while the slow-release properties of nanomaterials allow JQ1 to act on OSCC cells for a longer period of time. In addition, PEG-PLA nanoparticles were constructed to encapsulate and deliver JQ1. This synthesized nanoparticle is small in diameter and negative charge, which helps keep this nanoparticle stable in the bloodstream, as well as facilitating the drug act on tumor tissues through passive diffusion [36,37]. And meanwhile, the negative potential on the surface of PEG-PLA could minimize its own toxicity. What is more, in order to the drug to have a better targeting effect, we applied FA to modify this nanoparticle to treat OSCC on account of some previous findings that FR is highly expressed on the surface of OSCC cells, yet it is rarely expressed in normal cells [34]. However, FA-PEG-PLA-JQ1 showed a very good antitumor effect *in vitro*. We also found that phosphorylation-Forkhead box O1/Forkhead box O1 (p-FoxO1/FoxO1) and phosphorylation-protein kinase B/protein kinase

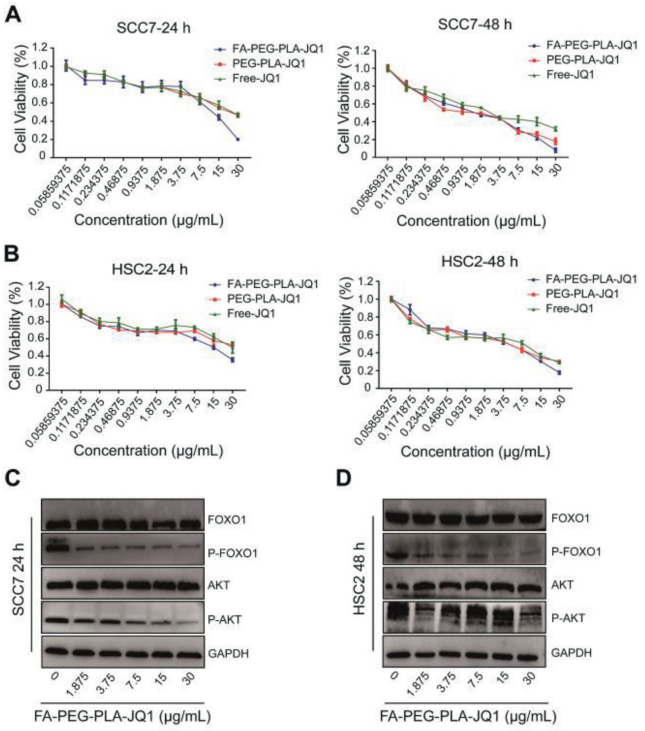


Fig. 2. The effects of FA-PEG-PLA-JQ1 on proliferation and cell cycle-related proteins on OSCC cells. (A, B) MTT assay was carried out in SCC7 cells (A) and HSC2 cells (B) treated with different drugs (Free-JQ1, PEG-PLA-JQ1 and FA-PEG-PLA-JQ1) for 24 h and 48 h [mean ± standard deviation (SD), $n = 5$]. (C, D) Western blot of cell cycle-related proteins on SCC7 cells (C) and HSC2 cells (D) after 24 h or 48 h with different concentration of FA-PEG-PLA-JQ1. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

B (p-Akt/Akt) show slight declines after treatment with FA-PEG-PLA-JQ1 (Figs. 2C and D).

Then, we evaluated the cell apoptosis of SCC7 cells and HSC2 cells through Annexin-fluoresceine isothiocyanate/propidium (FITC/PI) staining. After 48 h of 15 μg/mL FA-PEG-PLA-JQ1 treatment, the cell apoptosis percentage of SCC7 cells and HSC2 cells were 43.56% and 42.65% (Figs. 3A and B). We also examined some apoptosis-related proteins (Figs. 3C and D). The proportion of B-cell lymphoma-2 (BCL-2) to B-cell lymphoma-2 associated x protein (BAX) decreased with the increase of drug concentration in both SCC7 cells and HSC2 cells. In addition, FA-PEG-PLA-JQ1 could induce cleavage of caspase 3, caspase 8 and caspase 9.

The FR was expressed in SCC7 cells, which provided a basis for our subsequent experiments *in vivo* (Fig. 4A). All animal experiments were approved by the Animal Experimental Ethics Committee of State Key Laboratory Biotherapy (SKLB), Sichuan University. We established SCC7 subcutaneous tumor model on C3H/HeN mice to explore the anti-tumor effect of FA-PEG-PLA-JQ1 (Fig. 4B). Starting from day 9, PBS buffer, PEG-PLA, Free-JQ1, PEG-PLA-JQ1 and FA-PEG-PLA-JQ1 were injected into the body intravenously at the JQ1 dosage of 20 mg/kg every second day. On day 14, we executed all the mouses and excised their tumors. As shown in Figs. 4C and D, the average weight of these tumor tissues excised from NS, PEG-PLA, Free-JQ1, PEG-PLA-JQ1 and FA-PEG-PLA-JQ1 groups were 1.68, 1.44, 0.81, 0.28 and 0.06 g, respectively. The tumor weight in FA-PEG-PLA-JQ1 group was 15 times lighter than that of Free-JQ1 group and 5 times lighter than that of PEG-PLA-JQ1 group. And the tumor growth was slower in the drug treatment groups than this in the control group, especially in FA-PEG-PLA-JQ1 group (Fig. 4E). What is more, the C3H/HeN mice in FA-PEG-PLA-JQ1 group had no momentous change in body weight than mice in other groups (Fig. 4F). In addition, we also analyzed the biosafety of this drug

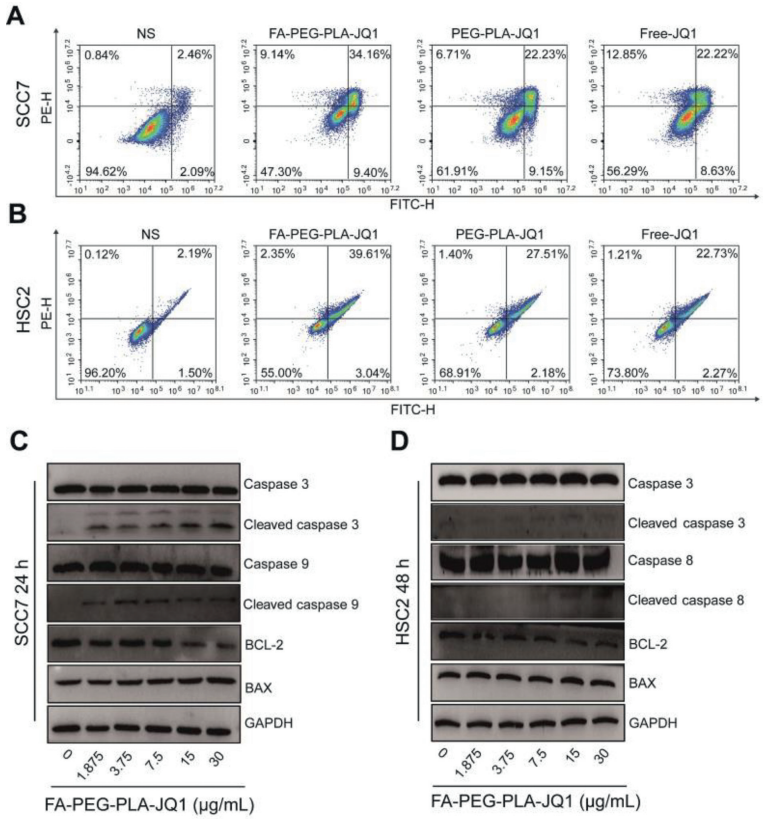


Fig. 3. The effects of FA-PEG-PLA-JQ1 on OSCC cells proliferation and apoptosis *in vitro*. (A, B) Flow cytometry detection of SCC7 cells (A) and HSC2 cells (B) apoptosis after different treatments for 48 h at the 15 μg/mL. (C, D) Expression of some apoptosis related proteins (BAX, BCL-2, caspase 3, cleaved caspase 3, caspase 8, cleaved caspase 8, caspase 9 and cleaved caspase 9) were detected by Western blot after treated with different concentration of FA-PEG-PLA-JQ1.

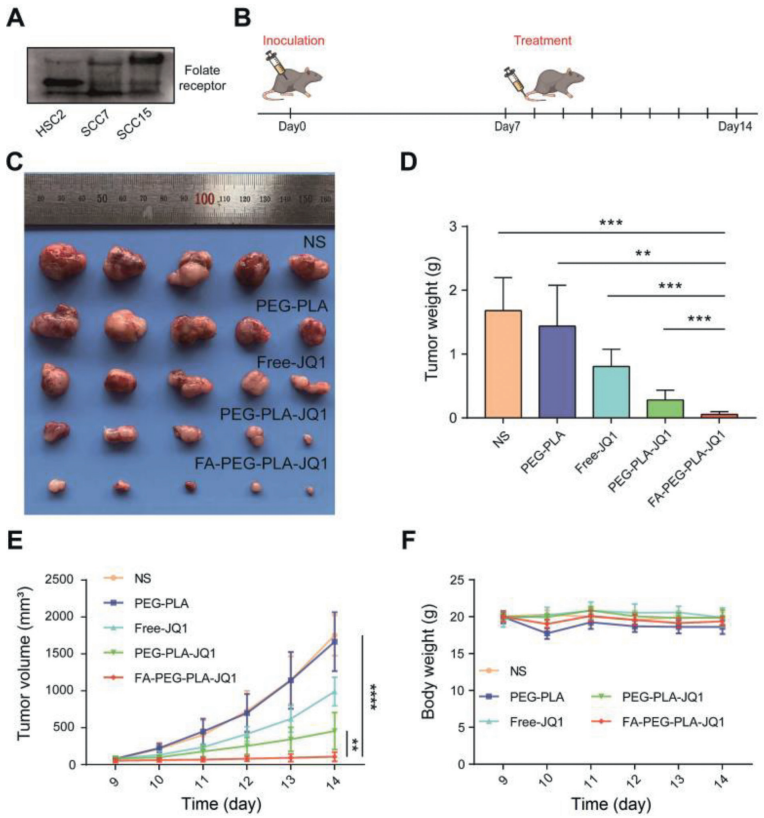


Fig. 4. *In vivo* evaluation of anti-tumor effects on SCC7 tumor-bearing mice. (A) Expression of folate receptor in OSCC cells. (B) Schematic of treatment design of subcutaneous model. (C) Photo of mice tumors in five groups: NS, PEG-PLA, Free-JQ1, PEG-PLA-JQ1, FA-PEG-PLA-JQ1, with 5 mice in each group. (D) Tumor weight of mice after different treatments (mean ± SD, $n = 5$). (E) Tumor growth curves in five groups (mean ± SD, $n = 5$). (F) Body weight in five groups (mean ± SD, $n = 5$). $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

delivery system. Tissue sections of vital organs (heart, liver, spleen, lung and kidney) were stained with hematoxylin-eosin (HE). As shown in Fig. S2 (Supporting information), there was no noticeable pathological changes in all groups. In addition, we also collected blood from different treated mice to measure certain vital biochemical markers and there were in normal range (Fig. S3 in Supporting information). These results confirmed that FA-PEG-PLA-JQ1 has the most outstanding anti-tumor effect than Free-JQ1 and PEG-PLA-JQ1, which could be because the FA-PEG-PLA was a passive and active targeting carrier, allowing more JQ1 to collect in the tumor site and then worked. As shown in Supporting information, we investigated the effect of FA-PEG-PLA-JQ1 on macrophage polarization, which might be a possible mechanism of anti-tumor drug.

In this study, a FA decorated tumor-targeted nanocarrier for JQ1 was designed successfully, which showed outstanding performance in tumor targeting and indistinctive toxicity for the treatment of OSCC. Through this drug delivery system, more JQ1 could be concentrated in tumor sites, thereby inhibiting tumor growth. Results revealed that tumor cells proliferation inhibition, tumor cells apoptosis induction, and M2 macrophages polarization attenuation might be the underlying mechanism for inhibitory effect of FA-PEG-PLA-JQ1 on OSCC. In summary, our work provided great potential for targeting delivery of JQ1 to tumor sites in OSCC treatment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.108433.

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