Archives of Oral Biology 92 (2018) 32–37

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Curcumin enhances anti-tumor immune response in tongue squamous cell carcinoma

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ARTICLE INFO

Keywords:
TSCC
4NQO mice model
Curcumin
PD-L1
Tregs
MDSCs

ABSTRACT

Purpose: This study evaluated the role of anti-tumor immune response of curcumin on tongue squamous cell carcinama (TSCC).

Experimental design: Cell lines (Cal 27, FaDu) and animal model (4NQO mice model) were uesd in this study. The MTT assay was used to detecte cell proliferation. The Western blotting, immunohistochemistry and immunofluorescence were used to examine the protein expression. The flow cytometry was performed to determine the number of Treg and MDSC.

Results: The expression of PD-L1 and p-STAT3^{Y705} were does-dependently inhibited in Fadu and Cal 27 cell line. The results of *in vivo* demonstrated that curcumin significantly attenuated tumor growth in 4NQO mice model. The expression of PD-L1 and p-STAT3^{Y705} were similarly decreased *in vivo*. Moreover, the anti-tumor immune response was remarkably improved after curcumin treatment through increasing CD8 positive T cells and decreasing Tregs and MDSCs.

Conclusions: Curcumin treatment resulted in inhibition of PD-L1 and p-STAT3^{Y705} expression both *in vitro* and *in vivo*. Moreover, the immunosuppressive tumor microenvironment was changed after curcumin treatment. These data suggested that curcumin could effectively promote anti-tumor immune response in TSCC.

1. Introduction

Oral cancer, with more than an estimated 500,000 newly diagnosed cases every year, is the sixth most common cancer in the world (Petersen, 2009). Tobacco, alcohol, HPV infection and physical irritation are the most risk factors for oral cancer (Jemal et al., 2011). And tongue squamous cell carcinoma (TSCC) is a major type of oral cancers. Even through current therapeutic strategies including surgery, radiation, chemotherapy and monoclonal antibody therapy, five year survival in only 40% due to the metastases and local occurrence (Mroz & Rocco, 2016). Host immune system plays a dual role in oral cancer development and malignancy (Economopoulou, Agelaki, Perisanidis, Giotakis, & Psyrri, 2016). There is an immuosuppressive status in the patients with oral cancer, such as superfluous regulatory T cells (Tregs) or myeloid derived suppressor cells (MDSCs) in patient's tumor microenvironment, over-expression of PD-L1 in cancer cells (Fugle et al., 2016; Weed et al., 2015; Zandberg & Strome, 2014). Therefore, now, oral cancer is considered as an immunosuppressive disease (Hirai et al., 2017).

To better explore the molecular mechanism for a kind of anti-tumor drug, a reliable animal model is critical (Mery et al., 2017). Knockout and transgenic mice have provided realistic models for oral cancer because of an intact immune system, especially for immunotherapy (Supsavhad, Dirksen, Martin, & Rosol, 2016). Besides, 4-nitroquinoline 1-oxide (4NQO), which severs as a surrogate of tobacco exposure, induced oral cancer mouse model is also an immunocompetent animal model (Ide et al., 2001). Moreover, this model closely mimics all carcinogenesis stages from various degrees of epithelial dysplasia to carcinoma (da Silva et al., 2017). It is worth noting that the increase of Tregs and MDSCs in peripheral blood and tumor draining lymph node is associated with tumor progress in 4NOQ-indeced TSCC mice model (Chu et al., 2012; Zhao et al., 2014).

Curcumin, the main bioactive compound found in the rhizome of *Curcuma longa L,* is identified to regulate different signaling pathways in several diseases (Kunnumakkara et al., 2017). Extensive studies over the last semicentury have identified that curcumin can both prevent and treat cancer, including oral cancer (Shehzad, Wahid, & Lee, 2010). Recent review demonstrated that curcumin is effective on oral cancer proliferation and survival, suggesting curcumin is a promising drug for oral cancer treatment (Borges et al., 2017). However, the mechanism of

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https://doi.org/10.1016/j.archoralbio.2018.04.015
Received 23 December 2017; Received in revised form 23 April 2018; Accepted 29 April 2018

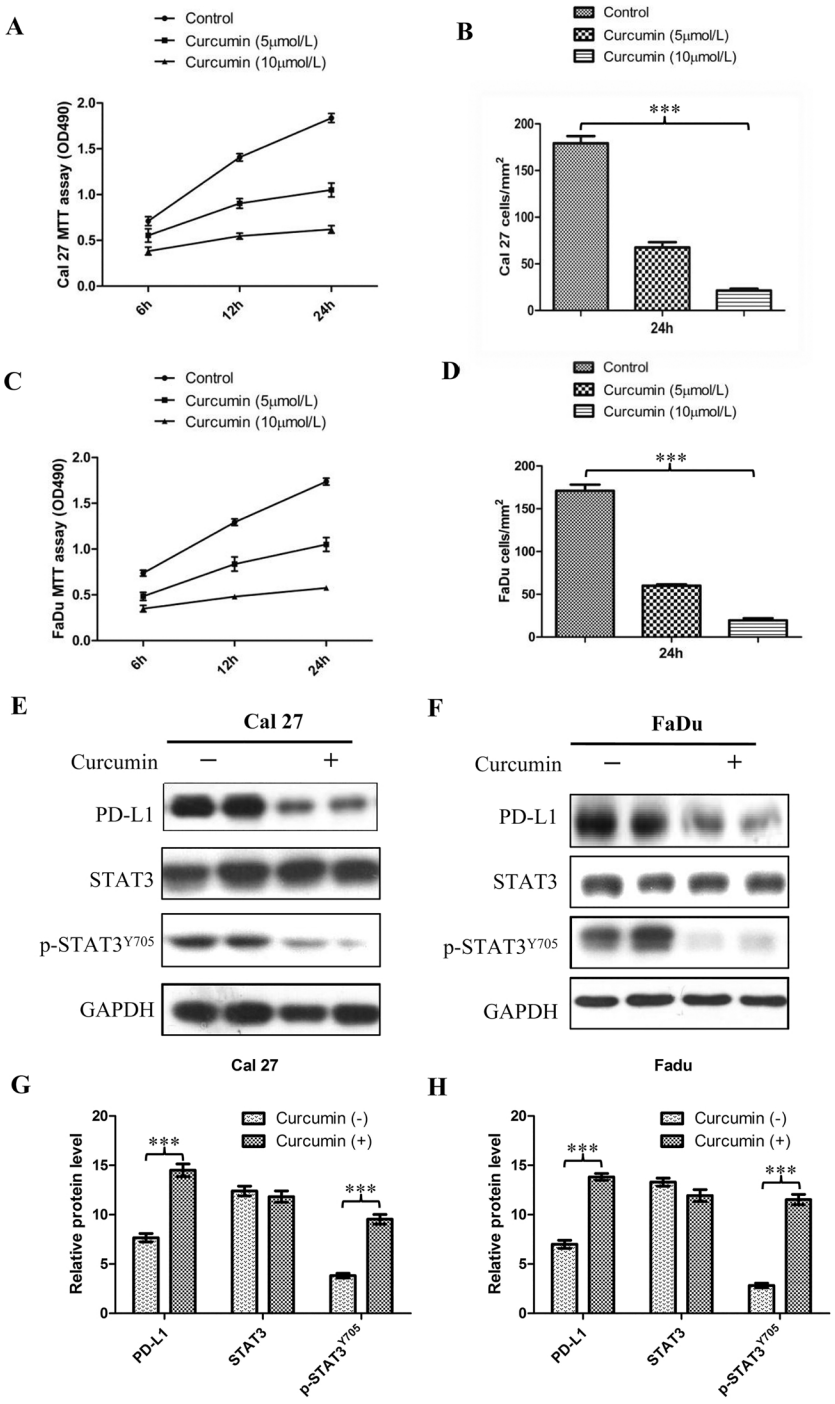

Fig. 1. Curcumin inhibited cell proliferation and reduced the expression of PD-L1 and p-STAT3 in OSCC cell line. A and B. Cal 27 cells were treated with different concentrations of curcumin (5 uM and 10 uM) or vehicle control DMSO for 6 h, 12 h and 24 h. The cell proliferation was determined by MTT assay. The data are represented as mean ± SEM (***, $p < 0.001$). C and D. Fadu cells were treated with different concentrations of curcumin (5 uM and 10 uM) or vehicle control DMSO for 6 h, 12 h and 24 h. The cell proliferation was determined by MTT assay. The data are represented as mean ± SEM. (***, $p < 0.001$). E. Western blot was used to analyze the protein counts of PD-L1, STAT3 and p-STAT3^{Y705} in Cal27 cell line with or without curcumin treatment. The GAPHD was used as loading control. F. Western blot was used to analyze the protein counts of PD-L1, STAT3 and p-STAT3^{Y705} in Fadu cell line with or without curcumin treatment. The GAPHD was used as loading control. G. Quantification of western blot in Cal27 cell line. The data are represented as mean ± SEM. (***, $p < 0.001$). H. Quantification of western blot in Fadu cell line. The data are represented as mean ± SEM. (***, $p < 0.001$).

curcumin for anti-tumor immune response remains unclear in TSCC.

PD-L1 (B7-H1 or CD274) and its receptor PD-1, play a critical role in the maintenance of immune homeostasis. In tumor microenvironment, cancer cells could utilize over-expression of PD-L1 to evade T cell-mediated tumor-specific immunity (Lin et al., 2018). The signal transducers and activators of transcription 3 (STAT3) involves in a variety of biological processes. In tumor, phosphorylation of STAT3 will lead to activation of downstream target genes, promoting tumor cell proliferation, autophagy, immune evasion and so on (Johnson, O'Keefe, & Grandis, 2018).

In this study, we examined the effect of curcumin on expression of PD-L1 and p-STAT3^{Y705} in cell line and 4NQO mice model. Further, we detected the regulation of curcumin for Tregs, MDSCs and T cells in 4NQO mice model.

2. Materials and methods

2.1. Cell culture

Cal 27 and Fadu cells were cultured with DMEM low-glucose medium supplemented with 10% fetal bovine serum and 100 U/ml penicillin and 100 μg/ml streptomycin in 37 °C, 95% humidified air and 5% CO_2 condition.

2.2. MTT assay

A total 3*10^4 cells per well were seeded onto fibronectin coated 24-well plates. 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay system (MTT) assay were performed according to the