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Triptolide induces mitochondria-mediated apoptosis of Burkitt's lymphoma cell via deacetylation of GSK-3β by increased SIRT3 expression

Jiamin Kong, Li Wang, Lu Ren, Yujing Yan, Yisen Cheng, Zhiying Huang*, Feihai Shen*

School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, PR China

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ABSTRACT

Burkitt's lymphoma (BL) is a highly aggressive B-cell non-Hodgkin lymphoma with rapid growth and dissemination propensity. Triptolide (TP), an active component extracted from Chinese herb *Tripterygium wilfordii* Hook f., has broad-spectrum anti-tumor activities. This study aimed to explore the *in vitro* and *in vivo* anti-cancer effects of TP on BL and the potential molecular mechanisms.

In this study, the *in vitro* anti-tumor activity of TP was determined by CCK-8 and flow cytometry assays in Raji, NAMALWA and Daudi cells. The expression of SIRT3, phosphorylation and acetylation of glycogen synthase kinase-3β (GSK-3β) were analyzed by Western blot assay. Moreover, we examined the mitochondrial membrane potential by JC-1 method and measured apoptosis related protein using Western blot assay. BL xenograft model in NOD/SCID mice were established to evaluate the *in vivo* anti-cancer effect of TP.

We discovered that TP inhibited BL cell growth and induced apoptosis in a dose-dependent manner. Loss of SIRT3 provides growth advances for BL cells. However, TP could up-regulate SIRT3 expression, which resulted in suppression of BL cells proliferation. GSK-3β was activated by SIRT3-mediated deacetylation, which subsequently induced mitochondrial translocation and accumulation of Bax and decrease of mitochondrial membrane potential. Anti-tumor studies *in vivo* showed that TP (0.36 mg/kg) inhibited the growth of BL xenografts in NOD/SCID mice with an inhibitory rate of 73.13%.

Our data revealed that TP triggered mitochondrial apoptotic pathway in BL by increasing SIRT3 expression and activating SIRT3/GSK-3β/Bax pathway. This study indicated that TP is a potential anti-cancer Chinese herbal medicine against BL.

1. Introduction

Burkitt's lymphoma (BL) is a highly aggressive B-cell non-Hodgkin lymphoma. The biologic hallmark of BL is a translocation activation and overexpression of *c-myc* gene (Dunleavy et al., 2016; Dozzo et al., 2017). The intensive chemotherapies with cyclophosphamide, doxorubicin and vincristine have been proven to be a promising therapy (Linch, 2012). However, the clinical outcome and prognosis of BL remain poor. Therefore, more novel therapeutic regimens and molecules of BL development are urgently needed for standard therapies.

Triptolide (TP), a diterpene triepoxide, was first isolated in 1972 from a traditional Chinese medicinal herb *Tripterygium wilfordii* Hook f. (Chemical structure in Fig. 1). It is clinically used to treat rheumatoid arthritis, psoriasis and lupus (Kupchan et al., 1972). In recent years, accumulated proofs show that TP has broad-spectrum anti-tumor properties, such as inducing cell cycle arrest and apoptosis of cancer cells. The underlying mechanism attracted much attention and has been studied intensively (Chan et al., 2001; Yang et al., 2003; Zhu et al., 2009; Li et al., 2011; Zhao et al., 2012; Li et al., 2014a). However, the efficacy of TP on BL therapy and its molecular mechanisms remain unclear.

SIRT3, a member of class III histone deacetylases (HDACs), is mainly located in mitochondria and involved in regulation of mitochondrial metabolism and oxidative stress (Giralt and Villarroya, 2012). Recent study indicated that loss of SIRT3 in B cell malignancies contributes to high invasiveness of BL. On the contrary, up-regulation of SIRT3 could induce cancer cell apoptosis, which suggests SIRT3 as a tumor suppressor (Kim et al., 2010; Yu et al., 2016). GSK-3β is a cytosolic signal molecule and functions in nuclei and mitochondria. It plays critical roles in cellular motility, metabolism and survival (Cohen and Frame, 2001; Sundaresan et al., 2015; Song et al., 2016). Previous studies suggested that GSK-3β activation by deacetylation subsequently

Abbreviations: TP, Triptolide; TWHF, *Tripterygium wilfordii* Hook f.; RSV, Resveratrol; DOX, Doxorubicin HCl; BL, Burkitt's lymphoma; GSK-3β, Glycogen synthase kinase-3β; Bax, BCL2-associated X protein; TUNEL assay, Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling assay

* Corresponding authors at: School of Pharmaceutical Sciences, 132 East Circle at Higher Education Mega Center, Guangzhou 510006, PR China.
E-mail addresses: hzhiying@mail.sysu.edu.cn (Z. Huang), shenfh3@mail.sysu.edu.cn (F. Shen).

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