DDIT4 is an Innate Protector in Psoriatic Keratinocytes and 1,25$(OH)_2D_3$ Exerts Anti-Psoriasis Through Promoting DDIT4 Inducing Macro-Autophagy and Proliferation Inhibition

Xiaojiao Zhang
First Affiliated Hospital of Chongqing Medical University
Fuling Luo
First Affiliated Hospital of Chongqing Medical University
Yi Luo
First Affiliated Hospital of Chongqing Medical University
Jing Li
Chongqing Key Laboratory of Biochemistry and Molecular Pharmacology, Chongqing Medical University
Jingyuan Wan
Chongqing Key Laboratory of Biochemistry and Molecular Pharmacology, Chongqing Medical University
LI Hu
First Affiliated Hospital of Chongqing Medical University
Hengguang Zhao (zhao@cqmu.edu.cn)
First Affiliated Hospital of Chongqing Medical University

Research Article

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Abstract

Psoriasis vulgaris is a chronic inflammatory skin disorder. Its pathogenesis is now still unelucidated and the treatment is far from satisfied. DNA Damage-Inducible Transcript 4 (DDIT4) is a widely expressed protein in different tissue, which can activate cell macro-autophagy through mTORC1 passway. Vitamin D_3 and analogues is a classic topical reagent for psoriasis vulgaris for more than 30 years, but its exact mechanism is not fully clear. In this study, we intend to verify whether vitamin D_3 also exerts anti-psoriasis through promoting DDIT4 inducing macro-autophagy and proliferation inhibition in psoriasis vulgaris. Results showed DDIT4 was over-expressed in psoriatic tissue, and probably acted as an innate protector during psoriasis pathogenesis. 1,25 dihydroxyvitamin D3 ($1,25(OH)_2D_3$) could promote DDIT4 expression in a rough linear correlation and subsequently activate macro-autophagy and inhabit cell proliferation, especially at the high concentration of 100nM. In terms of our understanding, this is the first time to reveal the interactions between $1,25(OH)_2D_3$, DDIT4 and macro-autophagy in psoriasis vulgaris. DDIT4 is also probably a potential therapeutic target in future psoriatic treatments.

Introduction

Psoriasis is a polygenic and inflammatory skin disease, there are several subtypes: psoriasis vulgaris, psoriasis anthropathica, psoriasis pustulosa and erythrodermic psoriasis. Psoriasis vulgaris is the most common type accounting for 90%. Numerous evidences show its pathogenesis including genetics such as HLA and MHC[1], environment, infection, injury, surgery, and so on. Accumulating studies have demonstrated that the distorted immune function and subsequent abnormality of keratinocytes proliferation and differentiation mediated the process of psoriasis[2].

Among the clinical treatment options for psoriasis, vitamin D_3 and its analogue is a classic reagent from about 30 years ago[3]. Topical vitamin D_3 (such as calcipotrol) is effective for psoriasis symptoms control, although its exact mechanism is still not fully elucidated. In our previous study, we confirmed that vitamin D_3 accelerates autophagy flux in human squamous-cell carcinoma (SCC) through DNA Damage-Inducible Transcript 4 (DDIT4) signal passway to exert its anti-tumor effect[4]. Like SCC, psoriasis vulgaris is an analogous benign over-proliferation disease, so we infer whether the same mechanism also occurs in the pathogenesis of psoriasis vulgaris.

DDIT4 is widely expressed in different tissue acting as a pivotal component under energy stress, wound healing and hair follicle differentiation[5]. Numerous studies have confirmed it is a crucial regulatory protein involved in malignant or over-proliferation diseases, such as breast cancer, prostate cancer and ovaries cancer[6–8]. As an evolutionarily conservative behavior, autophagy degrades intracellular metabolites and dysfunctional organelles for cellular survival. Autophagy is divided into three subtypes: macro-autophagy, micro-autophagy, molecular mediated autophagy. It is reported that macro-autophagy was usually initiated in some tumors or over-proliferation diseases, such as bladder cancer, prostate cancer, lung cancer, breast carcinoma, and human SCC[4,6–8]. However, little is known about the

contribution of DDIT4 and autophagy in psoriasis pathogenesis, so we hypothesized that DDIT4 and it induced macro-autophagy are both involved in the pathogenesis of psoriasis vulgaris and vitamin D_3 exert its anti-psoriasis effect through regulating DDIT4 related autophagy passway, moreover, DDIT4 and autophagy procedure are whether both potential therapeutic targets for future psoriasis treatment.

Materials And Methods

Tissue samples and immunohistochemistry

Skin psoriasis vulgaris samples and normal control tissues were obtained from patients with plaque-type psoriasis in Dermatological Department of the First Affiliated Hospital of Chongqing Medical University. The study was approved by the ethics committee of hospital and all the experiments performed were in accordance with the relevant guidelines and regulations. After written informed consents were signed, all the tissues were collected by surgery from the un-treated lesions. Especially, the normal control tissues were harvested from the surrounding margin of the psoriatic specimens. Formalin-fixed, paraffin-embedded tissues were stained by immunohistochemistry to detect the express of DDIT4 with anti-DDIT4 antibody (1:200, 5μg/ml, Abcam, USA). Average optical density (AOD) value of images were measured by Image-Pro Plus 6.0.

Primary psoriatic keratinocytes culture and treatments

To collect primary psoriatic cells, epidermis was separated from the dermis after 24h incubation with dispase II (Sigma-Aldrich, USA). Then the epidermal layer was separated, and single cells were incubated with 95%O_2, 5%CO_2 and DMEM at 37℃. Once 60% confluence, cells were treated with different concentration of 1,25 dihydroxyvitamin D3(1,25$(OH)_2D_3$, Sigma-Aldrich, USA) at 0nM (vehicle), 1nM,10nM,100nM for 24h.

Western blotting staining

Collected tissues or treated cells were lysed by RIPA with phosphatase inhibitors. BCA Protein Kit (Beyotime Biotechnology, China) was used to test protein concentration. Separated protein was then under the procedure of loading and electrophoresis. After transferred onto PVDF membrane, membrane containing protein was incubated with corresponding antibody and imaging was gotten. Antibodies including rabbit anti p4E-BP1 (Ser65, 1:1000, Cell Signaling Technology, USA), rabbit anti pS6K1 (Thr398, 1:1000, Cell Signaling Technology, USA), mouse anti p62 (1:2000, BD, USA), mouse anti DDIT4 (1:1000, Abcam, USA), rabbit anti Beclin1(1:1000, Cell Signaling Technology, USA), rabbit anti LC3B(1:1000, Sigma-Aldrich, USA) were used in this experiment. Bands were analyzed by image J software (Bethesda, MA, USA).

Transmission electron microscopy (TEM)

Cells were digested by trypsin, washed by PBS and fixed by stationary liquid for overnight at 4℃. Images were attained through the standard procedures including dehydration, embedding, solidification, section and imaging by TEM (Hitachi-7500, Japan).

Statistical analysis

Student's *t* test or One-way ANOVA were used for statistical analyses. Logest linear regression was used for correlation analysis. Statistical significance was considered when *p* value□0.05.

Results

1. DDIT4 was over-expressed in psoriatic lesions

DDIT4 expressions in psoriatic tissue and normal control were analyzed by immunohistochemistry. Results revealed DDIT4 was obviously over-expressed in psoriatic lesion than that in normal tissue (Fig.1A). Furtherly, DDIT4 mainly distributed in the cytoplasm and nucleus in the epidermal keratinocytes. In psoriatic specimen, DDIT4 positive cells mainly located in the basal cell layer and stratum spinosum, while in the normal tissue, it mainly concentrated only in the base call layer. These results not only indicated the featured accelerated proliferation in psoriasis keratinocytes, but DDIT4 probably also act as a meaningful protective role during the psoriasis pathogenesis attempting to repair the damaged DNA inside the fast proliferating keratinocytes of basal cells and stratum spinosum cells, which is identical with our previous finding in another analogous over-proliferative benign disease of human actinic keratosis (AK)[3].

To further verify this expression pattern of DDIT4, we performed Western blotting staining to quantitatively investigate DDIT4 expressions. Western blotting results verified DDIT4 was identically highly expressed in psoriatic tissue than that of normal control (Fig.1B and 1C).

2. 1,25$(OH)_2D_3$ promoted DDIT4 expression in primary psoriatic keratinocytes

To verify whether vitamin D_3 or its analogue could intervene DDIT4 expression in psoriatic tissue, different concentration of 1,25$(OH)_2D_3$ (0nM, 1nM, 10nM, 100nM) was administrated into the cultured primary psoriatic keratinocytes. After 24h incubation, DDIT4 expression was detected by Western blotting staining. Results showed that 1,25$(OH)_2D_3$ significantly promoted DDIT4 expression in cultured primary psoriatic keratinocytes (Fig.2A). It was consistent with our previous studies in human SCC and AK cells[3]. Among which, DDIT4 expression was most significantly induced by the highest concentration of 100nM 1,25$(OH)_2D_3$, and an approximate linear correlation was statistically revealed by Logest linear regression (Fig.2B).

3. 1,25$(OH)_2D_3$ promoted macro-autophagy through DDIT4-mTORC1 passway

DDIT4 is a widely accepted activator for mTORC1 induced macro-autophagy, which promotes autophagy flux through mTORC1 passway[9,10], and autophagosome formation is a featured event during autophagy flux. Then autophagosome formation was detected by TEM to quantitively evaluate the autophagy activity under different concentration $1,25(OH)_2D_3$. Results revealed all the investigated concentration of $1,25(OH)_2D_3$ could promote the autophagosome formation, also with the highest vertex at 100nM (Fig.3A and 3B), which suggested $1,25(OH)_2D_3$ enhanced the autophagy flux in psoriatic keratinocytes.

By Western blotting, we further investigated the autophagy related proteins expressions of P62, Beclin1, LC3 Ⅱ/Ⅰ(Fig.3C□3F). Results showed P62 expression was significant suppressed by $1,25(OH)_2D_3$ administration, but Beclin1 expression and LC3 Ⅱ/Ⅰ ratio were obviously elevated by $1,25(OH)_2D_3$. Identically, the highest vertex was also at the 100nM group.

4. $1,25(OH)_2D_3$ suppressed psoriatic keratinocytes proliferation through DDIT4 induced mTORC1 passway

4E-BP1 and S6K1 are two critical proliferation regulators inside DDIT4 induced mTORC1 passway, which can regulate cell proliferation through regulating mTORC1 mediated mRNA translation activity[11]. To further verify whether 4E-BP1 and S6K1 were intervened by $1,25(OH)_2D_3$, p4E-BP1(Ser65) and pS6K1(Thr389) were investigated by Western blotting under different concentration of $1,25(OH)_2D_3$ administration (Fig.4A□C). Results showed p4E-BP1 was significantly elevated while pS6K1 expression was significantly suppressed by all concentration of $1,25(OH)_2D_3$. Conclusively, it suggested that $1,25(OH)_2D_3$ also directly inhibited psoriatic keratinocyte proliferation through DDIT4 induced mTORC1 passway.

Discussion

Psoriasis vulgaris is a chronic, immune-mediated, polygenic skin disorder, which has serious influence on patient's quality of life. Although the pathogenesis of psoriasis vulgaris is still not fully elucidated, a large number of data confirmed that T lymphocytes such as Th-17 cell, Th-22 cell, regulatory T lymphocyte, and immune factors such as IL-17, IL-23, IL-22, IL-10, TNF-α, TGF-β were involved in the pathogenesis of psoriasis vulgaris[12]. Distorted immune function and the consequent abnormalities of cell proliferation are pathophysiological features of psoriasis vulgaris. Treatments, according to the subtype and psoriasis area and severity index (PASI) score, are often concentrated on topical medicine (vitamin D_3, glucocorticoids, etc.), oral antiproliferative drugs (ciclosporin, methotrexate, acitretin, etc.)[13]and biological agents (anti-tumor necrosis factor such as certolizumab, etanercept[14], IL-23 monoclonal antibody as guselkumab[15], IL-17 monoclonal antibody as secukinumab[16] Among which, vitamin D_3 and its analogues (for example, calcipotriol) is one the classic topical anti-psoriasis reagents, which was clinically used since 31 years ago[3], but its exact mechanism of action is still unknown.

Our previous study confirmed that vitamin D_3 could improve DDIT4 expression in human SCC and exert its anti-tumor effect via promoting the autophagy flux[4]. Therefore, we hypothesized whether the same mechanism also occurs in the pathogenesis and treatment of psoriasis vulgaris. In this study, we found that DDIT4 expression was significantly over-regulated in psoriasis vulgaris lesions compared with that of normal controls. This result may not only indicate that DDIT4 is a biological marker and innate protector during psoriasis pathogenesis, consistent with our previous findings in another benign over-proliferation disease of human AK[4], but also promoting DDIT4 expression may be a potential therapeutic target for psoriasis vulgaris.

Thereafter, cultured primary psoriasis keratinocytes were intervened by different concentration of $1,25(OH)_2D_3$ to investigate the effect of $1,25(OH)_2D_3$ on DDIT4 expression *in vitro*. A roughly linear correlation was verified between $1,25(OH)_2D_3$ concentration and DDIT4 expression. After DDIT4 activation, accelerated formation of autophagosome under $1,25(OH)_2D_3$ administration, as well as elevated Beclin1 and LC3 II/I ratio, but decreased P62 expression indicated the accelerated autophagy flux by $1,25(OH)_2D_3$ in primary cultured psoriatic keratinocytes. Meanwhile, increased phosphorylation of proliferation regulating proteins of 4E-BP1 and decreased phosphorylation of S6K1 suggested the inhibited cell proliferation ability under $1,25(OH)_2D_3$ administration through activating DDIT4 induced mTORC1 passway. Furthermore, considering the iconic role of LC3 II/I during the autophagy flux, the only existing statistical significance of LC3 II/I ratio between 100 nM $1,25(OH)_2D_3$ and all other groups (0 nM, 1 nM, 10 nM) (Fig. 3F) probably suggest only a high enough concentration can effectively interfere the autophagy procedure and exert its therapeutic effect on psoriatic vulgaris patients.

Conclusively, this research is the first time to demonstrate DDIT4 was involved in psoriasis vulgaris pathogenesis, and vitamin D_3 and its analogue exerted the anti-psoriasis effect through accelerating the DDIT4-mTORC1 mediated macro-autophagy flux and inhibiting cell proliferation by activating DDIT4 induced mTORC1 passway in psoriatic keratinocytes. However, more data should be collected in future for more details.

Declarations

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Availability of data and materials

all data generated and/or analyzed during the current study are included in this published article

Authors' contributions

Xiaojiao Zhang, Yi Luo, Jing Li and Li Hu performed the research. Hengguang Zhao and Jingyuan Wan designed the research study. Hengguang Zhao and Fuling Luo analyzed the data. Xiaojiao Zhang, Hengguang Zhao and Fuling Luo wrote the paper. All authors reviewed the manuscript

Ethics approval and consent to participate

The present study was approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University of Chinese Medicine; patients provided written informed consent.

Patient consent for publication

no applicable

Competing interests

The authors declare that they have no competing interests

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Figures

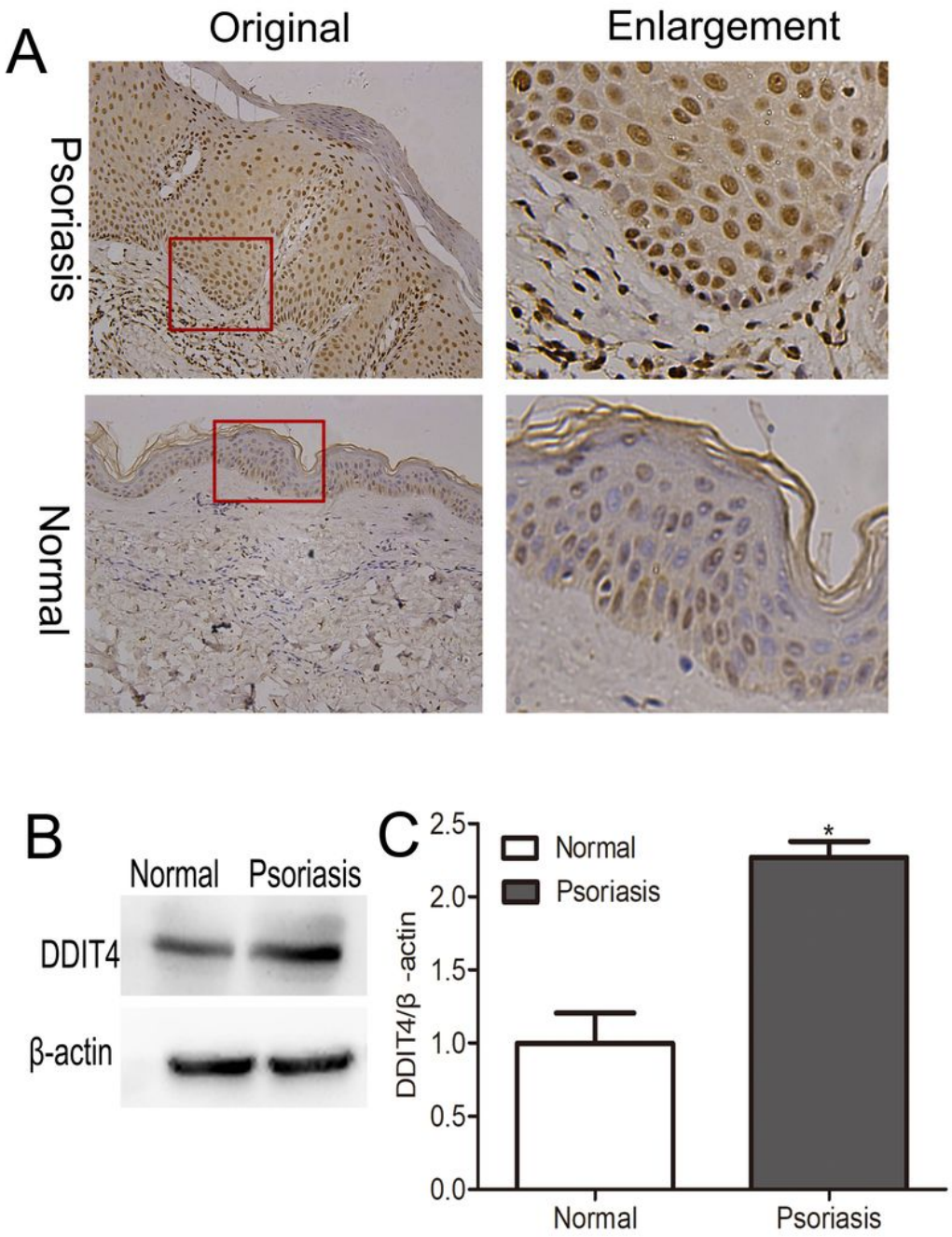

Figure 1

DDIT4 expression was over-expressed in psoriatic lesions A. IHC staining for DDIT4 in psoriatic lesions and normal controls. DDIT4 expression is significantly over-expressed in psoriatic tissue, especially inside the basal layer and stratum spinosum keratinocytes (IHC, ×100). B and C. DDIT4 expressions were detected by Western blotting. Blots cropped from different parts of the same gel. Values are shown as M±D. *, P□0.05.

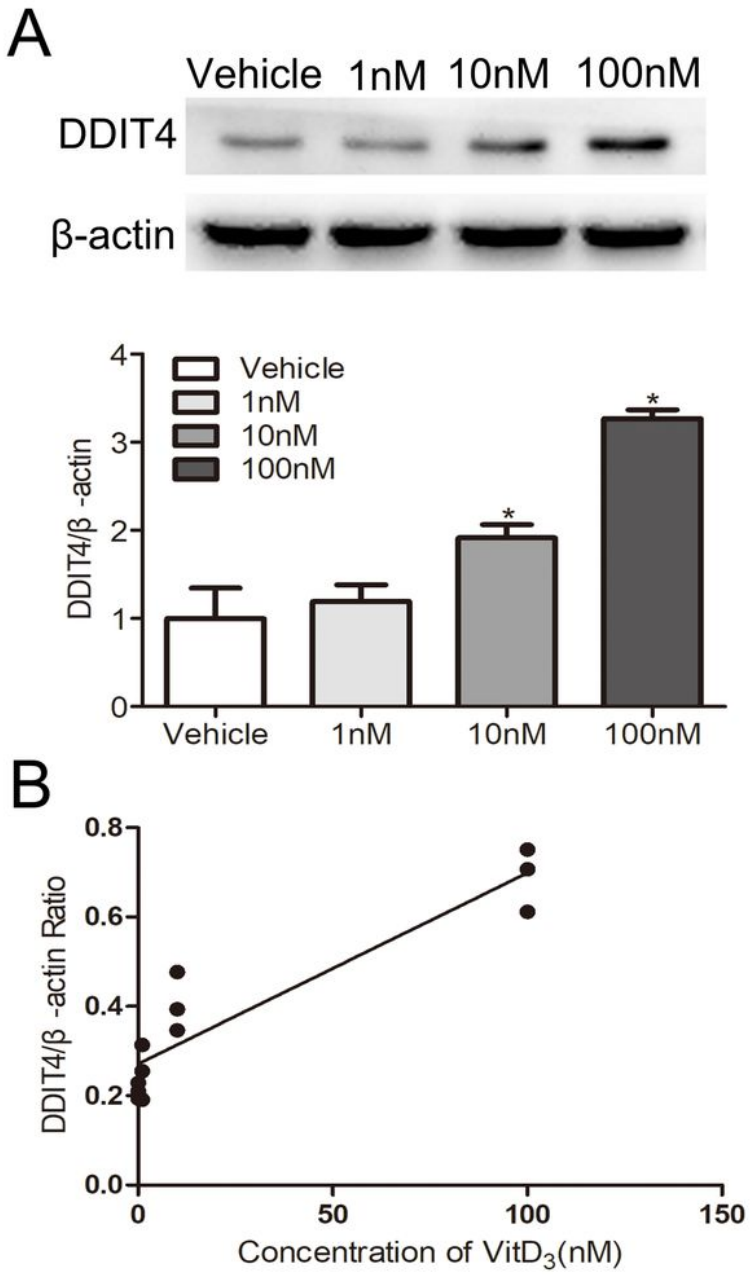

Figure 2

1,25(OH)2D3 promoted DDIT4 expression in primary psoriatic keratinocytes A. DDIT4 expression was significantly promoted by 1,25(OH)2D3 detected by Western blotting in cultured primary psoriatic keratinocytes. B. An approximate linear correlation was statistically revealed by Logest linear regression. Blots cropped from different parts of the same gel. *, P□0.05.

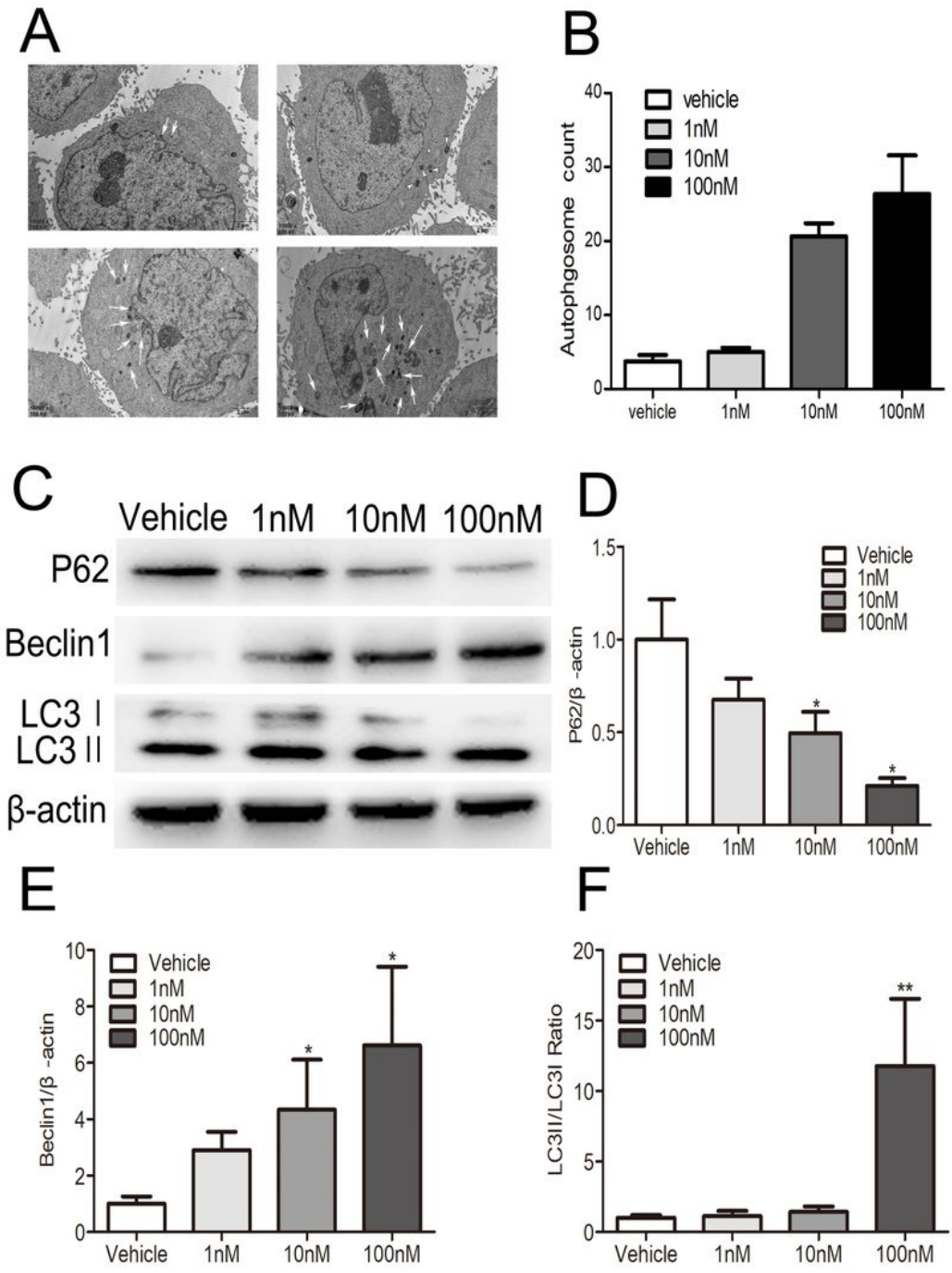

Figure 3

1,25(OH)2D3 promoted macro-autophagy through DDIT4-mTORC1 passway A and B. Autophagosome formations detected by TEM under different concentration 1,25(OH)2D3 administration. C□F. Autophagy related proteins expressions of P62, Beclin1, LC3 □/□were detected by Western blotting under different concentration of 1,25(OH)2D3 administration. Blots cropped from different parts of the same gel. *, P□ 0.05.

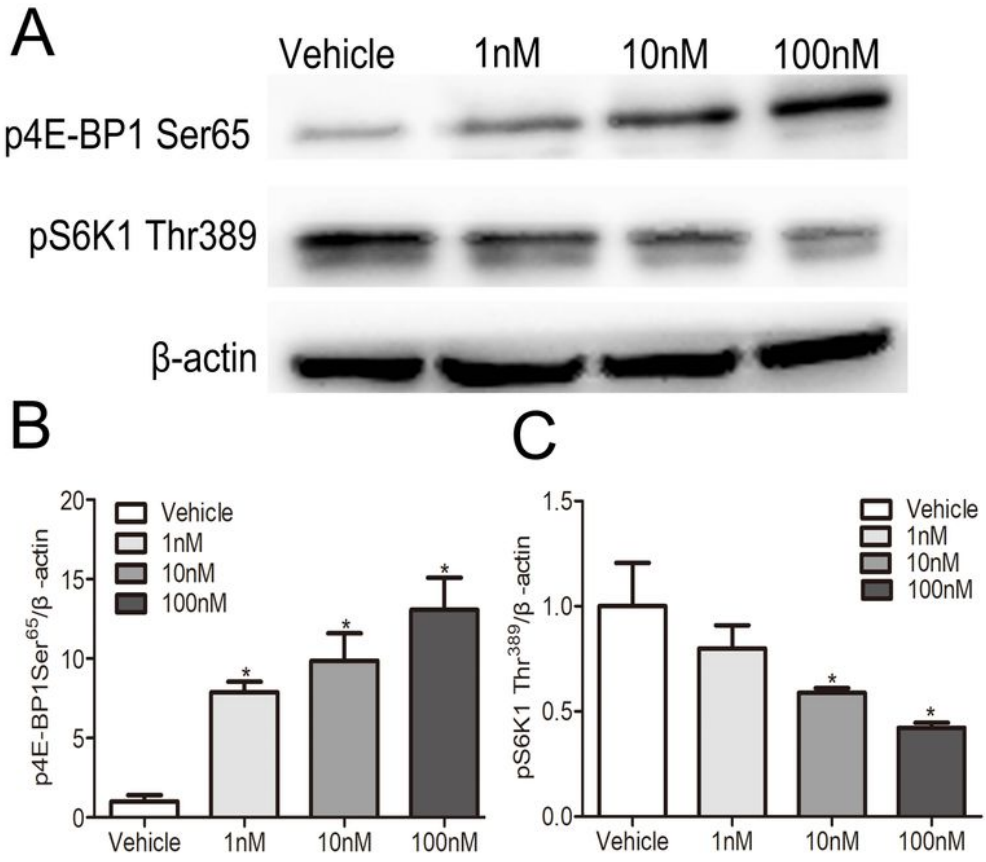

Figure 4

1,25(OH)2D3 suppressed psoriatic keratinocytes proliferation through DDIT4 induced mTORC1 passway. A□C. p4E-BP1(Ser65) and PS6K1 (Thr389) expressions were investigated by Western blotting under different concentration of 1,25(OH)2D3 administration. Blots cropped from different parts of the same gel. *, P□0.05.