Dose-specific effects of tumor necrosis factor alpha on osteogenic differentiation of mesenchymal stem cells

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Abstract

Objectives: To investigate tumor necrosis factor alpha (TNF- α)-induced changes in osteogenic differentiation from mesenchymal stem cells (MSCs).

Materials and methods: Blockade of nuclear factor- κ B (NF- κ B) was achieved in ST2 murine MSCs via overexpression of the NF- κ B inhibitor, I κ B α . Osteogenic differentiation was induced in I κ B α -overexpressing ST2 cells and normal ST2 cells when these cells were treated with TNF- α at various concentrations. Expression levels of bone marker genes were determined using real time RT-PCR and ALP activity assay. *In vitro* mineralization was performed to determine long-term exposure to TNF- α on mineral nodule formation. MTT assay was used to determine the changes in cell proliferation/survival.

Results: Levels of Runx2, Osx, OC and ALP were up-regulated in cell cultures treated with TNF- α at lower concentrations, while down-regulated in cell cultures treated with TNF- α at higher concentrations. Blockade of NF- κ B signaling reversed the inhibitory effect observed in cell cultures treated with TNF- α at higher concentrations, but showed no effect on cell cultures treated with TNF- α at lower concentrations. In contrast, long-term treatment of TNF- α at all concentrations induced inhibitory effects on *in vitro* mineral nodule formation. MTT assay showed that TNF- α inhibits proliferation/survival of mesenchymal stem cells when the NF- κ B signaling pathway is blocked.

Conclusions: The binding of TNF- α to its receptors results in the activation of multiple signaling pathways, which actively interact with each other to regulate the differentiation, proliferation, survival and apoptosis of MSCs.

Introduction

As the most common oral epidemics in adults, periodontal diseases are characterized by a specific inflammatory response to microbial residents of the subgingival biofilm, destroy tooth supporting alveolar bones, and yield tooth loosening and eventual tooth loss (1). Although it has been widely accepted that the plaque bacteria are the initial and necessary factor of periodontal diseases, emerging evidence has suggested that the inflammatory response of the host accounts for the majority of the hard- and soft-tissue damage observed in patients suffering from periodontal diseases (2). Therefore, it is of particular significance to thoroughly investigate the exact roles of host inflammatory responses in the pathogenesis of periodontal diseases, which presents the opportunity for developing new strategies for treating periodontal diseases by means of host response modulation (3).

Up till now, considerable research effort has been focused on the regeneration of the damaged periodontal tissues. In the 1980s, progenitor cells for cementum, bone, and periodontal ligament fibroblasts were found in the periodontal ligament tissue, and the regeneration of damaged periodontium can be achieved by the activity of local adult stem cells or progenitor cells found in paravascular locations of the periodontal ligament (4). Using a transgenic mouse model, we found that systemically transplanted bone marrow stromal cells possess the potential to migrate to bone wound sites, differentiate into osteoblast progenitor cells, and participate in bone regeneration in orocraniofacial region (5). However, currently the regeneration of damaged periodontal tissue is still an exclusive goal of periodontal therapy and remains a subject of intense

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interest to the dentists and dental scientists. In addition, the impact of inflammatory responses observed in the periodontal diseases on the migration, proliferation and differentiation of mesenchymal stem cells is largely unknown.

As the result of the activation of host-derived immune and inflammatory response to exogenous pathogens, pro-inflammatory cytokines are produced which significantly amplify the inflammatory response, lead to the production of lytic enzymes and stimulate the production of chemokines (3). Tumor necrosis factor alpha (TNF- α) is a key pro-inflammatory cytokine produced upon periodontal inflammatory response (6), which actively participates in osteoclastogenesis and tissue destruction observed in periodontal diseases (7). However, although it has been fully elucidated that TNF- α potently enhances osteoclastogenesis, the exact role of this cytokine in osteogenic differentiation from the mesenchymal stem cells is still ambiguous. In the murine myoblastic C2C12 cells, TNF- α suppresses BMP-2-induced expressions of runt-related transcription factor 2 (Runx2) and osteocalcin, which is mediated by the activation of NF- κ B signaling pathway (8) and can be reversed by estradiol and dexamethasone treatment (9). The osteogenic differentiation from the mesenchymal stem cells (MSCs) is also suppressed by TNF- α , which is demonstrated by decreased levels of alkaline phosphatase (ALP), α 1(I) procollagen, Runx2 and osterix after TNF- α treatment (10,11). On the other hand, TNF- α was shown to stimulate the expression of tissue-non specific alkaline phosphatase by vascular smooth muscle cells (VSMCs), which resulted in the trans-differentiation of VSMCs into osteoblast-like cells (12). Moreover, continuous delivery of TNF- α was reported to dose-dependently stimulate osteogenic differentiation from rat MSCs seeded in 3-dimensional biodegradable electrospun poly(epsilon-caprolactone) scaffolds (13).

In this study, ST2 murine mesenchymal stem cells were induced to differentiate towards osteogenic lineage cells when simultaneously treated with TNF- α at various concentrations. The changes in expression levels of osteogenic transcription factors and bone marker genes were determined to evaluate the effect of TNF- α on the osteogenic differentiation of these mesenchymal stem cells. The role of NF- κ B signaling pathway in TNF- α -mediated changes during osteogenic differentiation was also investigated to provide a novel explanation to the contradictory data from the literature review.

Materials and methods

Cell culture

The retroviral packaging cell line PT67 (Clontech, Palo Alto, CA, USA) and the murine mesenchymal stem cell

line ST2 (Riken Bio-Resource Center, Tsukuba, Ibaraki, Japan) were maintained in Dulbecco's Modified Eagle Medium (DMEM; HyClone, Logan, UT, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; HyClone), 100 U/ml penicillin (Invitrogen, Carlsbad, CA, USA) and 100 μ g/ml streptomycin (Invitrogen). For osteogenic differentiation, the cells were cultured in α -MEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μ g/ml streptomycin, 10^{-2} M β -glycerophosphate and 10^{-4} M ascorbic acid as described previously (14).

Production of viral stocks and viral infection

The retroviral vector encoding the mutant form of the NF- κ B inhibitor I κ B α (pBABE-puro-I κ B α -mut) was purchased from Addgene (plasmid ID: 15291, Cambridge, MA, USA). In this mutant form of I κ B α (I κ B α -mut), the alanine residues 32 and 36 are substituted for serine residues 32 and 36, which effectively prevent I κ B phosphorylation and degradation, and therefore constitutively prevent NF- κ B activation. Retroviral stocks containing pBABE-puro-I κ B α -mut or the empty viral vector pBABE-puro were prepared as previously stated (15). Briefly, pBABE-puro-I κ B α -mut or pBABE-puro was transfected into PT67 packaging cells using LipofectamineTM 2000 (Invitrogen). Forty-eight hours after the transfection, the supernatant of the transfected PT67 cells was collected, filtered through a 0.45 μ m filter, and stored at -80°C . The viral titer, 3×10^6 cfu/ml, was determined indirectly using NIH3T3 cells as previously described (15). For cell infection, ST2 cells were seeded on six-well plates at a cell density of 3×10^3 cells per plate, and incubated with viral stocks containing pBABE-puro-I κ B α -mut or pBABE-puro in the presence of polybrene (8 μ g/ml; Sigma, St. Louis, MO, USA) for 8 h. Forty-eight hours after the infection, the ST2 cells were subjected to puromycin selection (3 μ g/ml; Invitrogen) for 2 weeks. Stably transduced cells were used for the following experiments.

Western blot analysis

Whole protein lysates were prepared as described previously (16). Nuclear extracts were purified using a Nuclear Extraction Kit (Millipore, Billerica, MA, USA). SDS-PAGE and Western blot analyses were then performed using NuPAGE 4–12% Bis-Tris gradient gels and 0.45 μ m Invitrolon polyvinylidene fluoride membranes (Invitrogen) (16). Antibodies for I κ B α (1:500), NF- κ B subunit p65 (1:500), Lamin B1 (1:500) and β -actin (1:500) were purchased from Sigma. The secondary antibodies were horseradish peroxidase (HRP)-linked goat-anti rabbit IgG (Santa Cruz, Santa Cruz, CA, USA).

Blots were visualized using ECL chemiluminescence reagents from Pierce Biotechnology (Rockford, IL, USA). Images of blots were analysed using JD801 IMAGE 4.11 program (Dahui Biotechnology, Guangzhou, China).

Real-time reverse-transcriptase polymerase chain reaction

The stably transduced ST2 cells were induced to differentiate towards osteoblasts with the aforementioned osteogenic medium, which was supplemented with murine TNF- α (PeproTech Inc., Rocky Hill, NJ, USA) at different concentrations of 0, 0.01, 0.1, 1, 10 and 100 ng/ml, respectively. Forty-eight hours after the treatment, the cells were collected and subjected to real-time RT-PCR analysis as previously described (15). Briefly, total RNA was extracted using RNeasy Mini RNA Isolation kit (Qiagen GmbH, Hilden, Germany) and reverse transcribed to cDNA using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Shenzhen, China). Real-time PCR was performed using the LightCycler FastStart DNA Master SYBR Green I (Roche Applied Science, Penzberg, Germany). The sequences of the primers for amplification of mouse Runx2, osterix (Osx), osteocalcin (OC), ALP and GAPDH were as following: Runx2: 5'-TGTTCTCTGATCGCTCAGTG-3' and 5'-CCTGGGATCTGTAATCTGACTCT-3'; Osx: 5'-ATGGCGTCCTCTCTGCTTG-3' and 5'-TGAAAGGTCAGCGTATGGCTT-3'; OC: 5'-AAGCAGGAGGGCAATAAGGT-3' and 5'-TAGGCGGTCTCAAGCCAT-3'; ALP: 5'-AATGGGCGTCTCCACAGTAAC-3' and 5'-CTGAGTGGTGTTCATCGC-3'; and GAPDH: 5'-TGACCACAGTCCATGCCATC-3' and 5'-GACGGACACATTGGGGGTAG-3'. The amount of mRNA was calculated for each sample based on the standard curve using the LightCycler Software 4.0 (Roche). GAPDH was used as an internal control.

Alkaline phosphatase activity assay

The infected ST2 cells were seeded on 96-well plates at a cell density of 2×10^3 cells/well and incubated with the osteogenic medium supplemented with murine TNF- α at concentrations of 0, 0.01, 0.1, 1, 10 and 100 ng/ml, respectively. Forty-eight hours after the treatment, ALP activity was determined in these cells as an early marker of osteogenic differentiation as described previously (17). Briefly, an established colorimetric assay was used based on the rate of the conversion of a colorless substrate, p-nitrophenyl phosphate disodium salt hexahydrate (Sigma), to a yellow product, p-nitrophenol. Samples were run in triplicate, and the absorbance at 405 nm was measured.

In vitro mineralization assay

Infected ST2 cells were incubated with the above-mentioned osteogenic media supplemented with murine TNF- α at concentrations of 0, 0.1, 1 and 100 ng/ml, respectively. Four weeks after the osteogenic induction, *in vitro* mineralization was monitored using Alizarin red staining as described previously (15). The nodules were photographed with Olympus camera and the area covered with mineral nodules in each well was determined using image analysis system DT2000 software V1.0 (Tansi Technology Co. Ltd., Nanjing, China).

MTT assay

The stably transduced ST2 cells were seeded on 96-well plates at a cell density of 2×10^3 cells/well and treated with murine TNF- α at concentrations of 0, 0.01, 0.1, 1, 10 and 100 ng/ml, respectively. At 24, 48 and 72 h after the TNF- α treatment, the proliferation/survival of the cells was evaluated using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) test. Briefly, the culture medium was replaced with 5 mg/ml MTT solution (Beyotime, Shanghai, China) in PBS, and the plates were incubated for 5 h at 37 °C. The precipitate was extracted with DMSO (Sigma) and the optical density was measured at the wavelength of 490 nm.

Statistical analysis

Data were shown as means $\pm$ SEM from at least three experiments and *t*-test was used to test significance using SPSS 12.0 software (SPSS Inc., Chicago, USA). $P < 0.05$ was considered statistically significant.

Results

The NF- κ B signaling pathway was successfully inhibited via the overexpression of the NF- κ B antagonist I κ B α

The ST2 cells stably transduced with pBABE-puro-I κ B α -mut or pBABE-puro were collected and subjected to western blot analysis to evaluate the cytosolic protein level of I κ B α . As shown in Fig. 1A, the protein level of I κ B α was increased by 2.8 folds in ST2 cells transduced with pBABE-puro-I κ B α -mut when compared with the control ST2 cells (Fig. 1A). As a result, the nuclear protein level of p65, a subunit of NF- κ B, was slightly decreased in ST2 cells stably overexpressing the exogenous I κ B α when compared with the control cells (Fig. 1B). After treated with 100 ng/ml TNF- α , the nuclear protein level of p65 was dramatically increased in the control ST2 cells, while in the TNF- α -treated

Figure 1. The NF- κ B signaling pathway was successfully inhibited via the overexpress of NF- κ B antagonist I κ B α . Western blot analysis was performed to determine the cytosolic protein level of I κ B α and the nuclear protein level of the NF- κ B subunit, p65, in the ST2 cells stably infected with pBABE-puro or pBABE-puro-I κ B α -mut. (A) Changes in the cytosolic protein level of I κ B α . Data were represented as mean $\pm$ SEM. *, $P < 0.05$, ST2-pBABE-puro-I κ B α -mut cells versus ST2-pBABE-puro cells. (B) Changes in the nuclear protein level of p65. a, $P < 0.05$, versus ST2-pBABE-puro cells treated without TNF- α ; b, $P < 0.05$, versus ST2-pBABE-puro-I κ B α -mut cells TNF- α .

ST2-pBABE-puro-I κ B α -mut cells, the increase in the nuclear protein level of p65 was less prominent (Fig. 1B).

Short-term TNF- α treatment resulted in dose-dependent changes in the expression levels of osteogenic transcription factors and bone marker genes during osteogenic differentiation

To investigate the role of TNF- α in the osteogenic differentiation of mesenchymal stem cells, ST2 murine mesenchymal stem cells were induced to differentiate towards osteoblasts and treated with TNF- α at various concentrations. Interestingly, real time RT-PCR analysis showed that the mRNA levels of the potent osteogenic transcription factors (Runx2 and Osx) and some bone matrix proteins (OC and ALP) were up-regulated in cell cultures treated with TNF- α at lower concentrations (0.01 and 0.1 ng/ml) when compared with cells treated with 0 ng/ml TNF- α . In contrast, the mRNA levels of Runx2, Osx, OC and ALP were dose-dependently down-regulated in cell cultures treated with TNF- α at concentrations above 1 ng/ml (Fig. 2a). Blockade of NF- κ B signaling via the overexpression of the NF- κ B antagonist I κ B α only reversed the inhibitory effect observed in cell cultures treated with TNF- α at concentrations higher than 1 ng/ml, while had no impact on the positive changes observed in cell cultures treated with TNF- α at lower concentrations (Fig. 2b).

Similarly, 48-h treatment of the ST2 cells with TNF- α at lower concentrations (0.01 and 0.1 ng/ml) enhanced ALP activity in these cells when compared with the control cells (treated with 0 ng/ml TNF- α). Cells treated with TNF- α at higher concentrations (10 and 100 ng/ml) showed decreased ALP activity when compared with the

control cells (Fig. 3a). Overexpression of I κ B α reversed the down-regulation of ALP activity by TNF- α treatment at concentrations of 10 and 100 ng/ml, while failed to induce significant changes in ALP activity of cells treated with TNF- α at lower concentrations (0.01 and 0.1 ng/ml) (Fig. 3b).

No significant difference was detected between ST2-pBABE-puro cells and ST2-pBABE-puro-I κ B α -mut cells which were treated without TNF- α .

Long-term treatment of TNF- α mainly induced inhibitory effects on in vitro mineral nodule formation

In vitro mineral nodule formation was induced in ST2 cells infected with pBABE-puro-I κ B α -mut or pBABE-puro using the above-mentioned osteogenic media for 4 weeks. At the same time, the cells were incubated with TNF- α at various concentrations for 4 weeks. This long-term treatment with TNF- α resulted in a dose-dependent decrease in mineral nodule formation after the 4-week osteogenic induction [Fig. 4a (upper panel),b]. The inhibitory effect of TNF- α on *in vitro* mineral nodule formation was partially reversed by the blockade of NF- κ B signaling [Fig. 4a (lower panel),c]. No significant difference was detected between ST2-pBABE-puro cells and ST2-pBABE-puro-I κ B α -mut cells which were treated without TNF- α .

TNF- α inhibits proliferation/survival of mesenchymal stem cells with the blockade of NF- κ B signaling pathway

MTT assay did not reveal any statistically significant changes in the proliferation/survival of ST2 cells treated

Figure 2. Effects of short-term TNF- α treatment on osteogenic gene expression in MSCs. ST2 cells stably infected with pBABE-puro (a) or pBABE-puro-I κ B α -mut (b) were induced to differentiate towards osteoblasts with the osteogenic medium, which was supplemented with murine TNF- α at concentrations of 0, 0.01, 0.1, 1, 10 or 100 ng/ml. Forty-eight hours after the treatment, real time RT-PCR was performed to determine mRNA levels of Runx2, Osx, OC and ALP in these cells. Data were represented as mean $\pm$ SEM. *, $P < 0.05$, versus cells treated with 0 ng/ml TNF- α .

with TNF- α at concentrations ranging from 0.01 to 100 ng/ml (Fig. 5a). Interestingly, the blockade of NF- κ B signaling pathway via the overexpression of I κ B α showed a dose-dependent negative effect of TNF- α on the proliferation/survival of ST2 cells (Fig. 5b). No significant difference was detected between ST2-pBABE-puro cells and ST2-pBABE-puro-I κ B α -mut cells which were treated without TNF- α .

Figure 3. Effects of short-term TNF- α treatment on ALP activity in MSCs differentiating towards osteogenic lineage cells. ST2 cells stably infected with pBABE-puro (a) or pBABE-puro-I κ B α -mut (b) were induced to differentiate towards osteoblasts with the osteogenic medium, which was supplemented with murine TNF- α at concentrations of 0, 0.01, 0.1, 1, 10 or 100 ng/ml. Forty-eight hours after the treatment, alkaline phosphatase (ALP) activity was determined using an established colorimetric assay. Data were represented as mean $\pm$ SEM. *, $P < 0.05$, versus cells treated with 0 ng/ml TNF- α .

Discussion

TNF- α is a 17-kDa cytokine produced by macrophages and other immune cells (18). It is the founding member of the TNF superfamily and has two different receptors: TNF receptor-1 (TNFR1) and TNF receptor-2 (TNFR2) (19). TNFR1 is expressed in almost all cells and is the functional form of the receptor in both osteoblasts and osteoclasts (20), whereas TNFR2 is primarily expressed in immune cells to regulate inflammation (18). In most cells,

Figure 4. Long-term treatment of TNF- α mainly induced inhibitory effects on *in vitro* mineral nodule formation. ST2 cells stably infected with pBABE-puro or pBABE-puro-I κ B α -mut were induced to differentiate towards osteoblasts with the osteogenic medium for 4 weeks. During the 4-week osteogenic induction, the cells were also incubated with murine TNF- α at concentrations of 0, 0.1, 1 or 100 ng/ml. Four weeks after the osteogenic induction, *in vitro* mineralization was monitored using Alizarin red staining. (a) Demonstrative photos of osteogenesis in ST2 cells stably infected with pBABE-puro (upper panel) or pBABE-puro-I κ B α -mut (lower panel). (b) Quantification of positively stained area by Alizarin red in ST2 cells stably infected with pBABE-puro. Data were represented as mean $\pm$ SEM. *, $P < 0.05$, versus cells treated with 0 ng/ml TNF- α . (c) Quantification of positively stained area by Alizarin red in ST2 cells stably infected with pBABE-puro-I κ B α -mut. Data were represented as mean $\pm$ SEM. *, $P < 0.05$, versus cells treated with 0 ng/ml TNF- α .

the binding of TNF- α to TNFR1 causes the formation of a complex by TNF receptor-associated protein with death domain (TRADD), receptor-interacting kinase (RIP), and TNF-receptor-associated factor 2 (TRAF2), which eventually activates NF- κ B signaling pathway and mitogen-activated protein kinase (MAPK) such as p38, ERK and JNK (21). The activation of multiple signaling pathways after TNF- α treatment and the interaction between these signaling pathways contribute to the complexity of TNF- α -induced effect on osteoblast differentiation, proliferation and apoptosis.

In this study, we found that TNF- α treatment at lower concentrations moderately enhanced expression levels of osteogenic transcription factors and bone marker genes. In contrast, TNF- α treatment at higher concentrations displayed inhibitory effect on osteogenic differentiation. Interestingly, the inhibitory effect of TNF- α observed in cell cultures treated with higher concentrations could be

reversed by the blockade of NF- κ B signaling pathway, while the overexpression of I κ B α had no effect on the positive effect of TNF- α treatment at lower concentrations. These results clearly indicated that different signaling pathways dominate in cell cultures treated with TNF- α at different concentrations. It has been reported that after TNF treatment, JNK activation was transient because the activation of JNK can be shut down by NF- κ B activation (22,23). In addition, the activation level of NF- κ B is dramatically enhanced with the increase in TNF- α concentration, while the activation level of members of the MAPK family including JNK, ERK and p38 only demonstrates a moderate increase or even moderate decrease with the increase in TNF- α concentration (24,25). While most studies have reported the inhibitory role of activated NF- κ B signaling pathway in osteogenic differentiation and bone formation (26,27), it was reported that JNK, ERK and p38 are capable of stimulating differentiation of

Figure 5. TNF- α inhibits proliferation/survival of MSCs with the blockade of NF- κ B signaling pathway. ST2 cells stably infected with pBABE-puro (a) or pBABE-puro-I κ B α -mut (b) were seeded on 96-well plates at a cell density of 2×10^3 cells/well and treated with murine TNF- α at concentrations of 0, 0.01, 0.1, 1, 10 or 100 ng/ml. At 24, 48, and 72 h after the TNF- α treatment, the proliferation/survival of the cells was evaluated using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) test. Data were represented as mean $\pm$ SEM.

osteoblasts (28–30). Together with these previous findings, our results suggest that the treatment with TNF- α at lower concentrations mainly activates the MAPK family members, which in turn displays positive effects on osteogenic differentiation. In contrast, higher concentrations of TNF- α result in the activation of NF- κ B signaling pathway, and as a result, the osteogenic differentiation is inhibited directly by the activated NF- κ B.

Our results also demonstrated that long-term treatment with TNF- α displays dose-dependent inhibition in osteogenic differentiation, which can be reversed by the suppression of NF- κ B signaling pathway. These data is consistent with previous findings indicating that prolonged TNF- α treatment usually results in prominent activation of NF- κ B, while the usually transiently activated

MAPK members such as JNK is rapidly inactivated by NF- κ B signaling (22,23). The enhancement of mineral nodule formation derived from the inactivation of NF- κ B signaling pathway is also consistent with previous findings showing that a reduction in NF κ B activity in osteoblasts results in an increase in bone formation via an increase in JNK activity (31).

The different signaling pathways activated by TNF- α stimulation also demonstrated divergent effects on cell proliferation, survival and apoptosis. For example, TNF- α induces the activation of the MAP kinase cascade including apoptosis signal-regulating kinase 1 (ASK-1), mitogen-activated protein kinase kinase 4/7 (MKK4/7) and JNK, which are all believed to mediate cell apoptotic and necrotic cell death (22,32,33). Conversely, TNF- α treatment simultaneously activate a survival pathway mediated by the activation of NF- κ B (18,33). The final cell response (proliferation versus apoptosis) to TNF- α treatment is thus determined by the balance between the apoptotic signaling pathway and the NF-kappaB survival pathway (19). Consistent with previous findings (10,34), we found intact cell proliferation/survival in mesenchymal stem cells after TNF- α treatment at concentrations ranging from 0.01 to 100 ng/ml. In addition, the inactivation of the cell survival pathway via the overexpression of the NF- κ B inhibitor resulted in a significant decrease in cell proliferation/survival.

In conclusion, the binding of TNF- α to its receptor results in the activation of multiple signaling pathways, which actively interact with each other to regulate the differentiation, proliferation, survival and apoptosis of mesenchymal stem cells. This makes it extremely challenging to find out the final role of TNF- α in osteogenic differentiation, bone formation and tissue regeneration, especially in the situation of periodontal diseases which are characterized by enhanced inflammatory response and fluctuating TNF- α level. Indeed, the effects of TNF- α and cell-signaling molecules on normal and pathological cellular processes still need further investigation to prevent excessive tissue destruction resulted from excessive production, dysregulation or inadequate inhibition of pro-inflammatory cytokines.

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