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Original Research Article

N-methyl-D-aspartate receptor together with complement C5 accelerated chondrocyte apoptosis and inflammation of osteoarthritis via mediating NLRP3

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Abstract

Purpose: *NMDAR and complements have been reported to facilitate progression of OA but the correlation between NMDAR and complement C5 in OA is unknown. Therefore, NMDAR antagonist, MK801 and C5 inhibitor, eculizumab were applied to measure functions of NMDAR and C5 in SW1353 cells.*

Methods: *SW1353 cells were first induced with LPS (5 μg/ml and 10 μg/ml) and later 10 μg/ml LPS induced SW1353 cells were treated by MK801 (100 μM) and eculizumab (100μg/ml). Thereafter, NR1, NR2A and C5 RNA expressions were assessed by RT-qPCR in LPS induced SW1353 cells and NR1 with NR2A was measured under MK801 treatment while C5 was analyzed by eculizumab treatment. Later, apoptosis of SW1353 cells were examined through flow cytometry. Inflammatory cytokines TNF-α, IL-6 and NLRP3 RNA expressions were evaluated by RT-qPCR in SW1353 cells from different groups.*

Results*: NR1, NR2A and C5 RNA expressions were all significantly upregulated after LPS induction with upregulated apoptosis and increased TNF-α, IL-6 and NLRP3. Treatment of MK801, a NMDAR antagonist, reduced NR1 and NR2A levels. Moreover, apoptosis rate of SW1353 and RNA expressions of inflammatory cytokines were all markedly reduced with MK801. Further, eculizumab treatment significantly downregulated C5 RNA expression in LPS induced SW1353 cells. Added eculizumab also enhanced functions of MK801 in repressing apoptosis rate and inflammatory factors RNA expressions.*

Conclusion*: MK801 and eculizumab suppressed apoptosis and inflammatory response in SW1353 cells through inhibiting NMDAR and complement C5.*

Keywords*: NMDAR, complement C5, NLRP3, osteoarthritis*

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INTRODUCTION

Osteoarthritis (OA) is the most commonly diagnosed chronic joint disease involving inflammatory process and other pathological changes, which is the main reason resulting in pain and disability of the aged [1]. OA can be caused by many reasons like age, joint injury,

obesity and so on [2,3] and over a tenth of the population aged older than 60 will develop clinical problems ascribed to osteoarthritis, leading to heavy burden of public health [4]. What's worse, because of aging of population and growing of obesity, occurrence rate of OA will grow gradually [5]. Essentially, OA is a kind of inflammatory disease[6]. During progression of OA, inflammatory response can be attributed to out of balance between inflammatory factors and anti-inflammatory cytokines [7].

Glutamate (Glu) is the main excitatory neurotransmitter through acting on Glu receptor in central nervous system. However, over activation of those receptors can cause damages and even death of nerve cells [8]. The N-methyl-d-aspartate (NMDA) receptor (NMDAR) is a receptor of glutamate that is widely distributed in brain and spinal cord [9]. Based on previous studies, NMDAR is essential to learning and synaptic plasticity and it can mediate death of neurons [10]. Moreover, NMDAR also expressed in other tissues such as renal, cardiovascular system, urinary system and so on [11,12]. In cells, activation of NMDAR caused Na+ and Ca2+ flowing into cells and overloaded Ca2+ resulted in apoptosis or necrosis of cells [13]. NMDAR contains three subunits: NR1, NR2 and NR3 and several different subtypes exists in NR2 (NR2A-D) and NR3 (NR3A and NR3B) [14]. In OA, NMDAR subunits were found to be expressed in OA chondrocytes [15] and NMDAR antagonist, MK801 blocked inflammation of SW1353 cells in a dose dependent manner [15].
Complement system is a representative part of innate immune system which can mediate many kinds of responses like inflammation reaction, cell lysis cessation and pathogen phagocytosis, etc.[16,17] Nevertheless, excessive activation of complements can lead to damages of tissues in diseases [17]. Activation of complements is transited by dissociation of C3 and further activation leads to lysis of C5 to C5a and C5b [18]. C5a is known pro-inflammatory cytokine and C5b induction to C5b-9 can promote inflammation through calcium influx [19,20]. In OA samples, C5 was upregulated, proving that complement C5 participated progression of OA [21]. Moreover, C5a and C5b-9 were both highly expressed in OA patients [22]. For retarding functions of complements, Eculizumab is acknowledged as the inhibitor of complement C5 which can block its activation for preventing cell injury resulted from complements mediated inflammation [23]. Though NMDAR and complement C5 were reported that either of them could accelerate progression of OA, their correlation was seldom mentioned. Therefore, we selected these two cytokines to study the mechanism beneath them in mediating OA progression and inflammation.

METHODS

Cell culture and treatment

SW1353, human chondrosarcoma cell line [24], was acquired from American Type Culture Collection (ATCC, USA) and incubated in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich, Germany) replenished with 10% fetal bovine serum (FBS, Sigma-Aldrich, Germany), 2 mM L-Glut and 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, USA) at 37 ℃ and 5% CO_2. SW1353 cells were then divided and treated for 12h with different concentrations of LPS (5 μg/ mL and 10μg/ mL) for next experiments [25]. Later, 10 μg/ mL LPS induced SW1353 cells were chosen to be treated with MK801 (100 μM) for 24h [26] and then disposed by 100 μg/ml eculizumab for 4h [18]. After LPS treatment, SW1353 cells in log phase were chosen.

RT-qPCR

After incubation and treatments of SW1353 cells, total RNA was extracted from SW1353 cells strictly according to manufacturer's instruction of TRIzol reagent (Invitrogen, USA). Thereafter, complementary cDNA was compounded through reverse transcription of RNA using SuperScript™ IV First-Strand Synthesis System from Invitrogen (USA). PCR detection then was performed to quantify relative RNA expressions with Fast SYBR™ Green Master Mix (Applied Biosystems, USA) and ProFlex™ PCR System (Applied Biosystems, USA). Sequences of primers were listed as follows: C5: forward, 5-GGCACAAAGTCCTCCAAATG-3 and reverse, 5'- CCAAACCAAGTCTCCAGTGA-3[27]; NR1: forward, 5'- GTCCACCAGACTGAAGATTGTG AC-3' and reverse, 5'- CTCCTCCTTGCATG TCCCA-3'[28]; NR2A: forward, 5'- GCTCTTC TCCATCATCAGCAGGG -3' and reverse, 5'-GGATCCCGTCAGATTGAAGTCT -3' [28]; NLRP3: forward, 5'- GGACTATTTCCCCAAGA TTG-3' and reverse, 5'- ACTCCACCCGAT GACAGTT-3' [29] and GAPDH: forward, 5'-GGCATGGACTGTGGTCATGAG -3' and reverse, 5' -TGCACCACCAACTGCTTAGC -3' [28]. For PCR amplification, 2.5 μL diluted cDNA and primers were added into 12.5 μL Fast SYBR™ Green Master Mix and then PCR was performed at ProFlex™ PCR System. Predenaturation was first at 95℃ for 10min and later denaturation was at 95℃ for 30s. Annealing was at 60℃ for 30s and extension was at 72 ℃ for 1 min, 40 cycles. These three transcripts were

measured in triplicate. Relative expressions of C5, NR1 and NR2A were calculated by $2^{-\triangle\triangle Ct}$ method normalizing to GAPDH.

Flow cytometry

For making apoptosis analysis, SW1353 cells in different groups were first digested with 0.25% trypsin and rinsed by PBS twice. Following, SW1353 cells were resuspended in 195 µl Annexin V-FITC binding buffer (Beyotime, Shanghai, China) with 5×10^4 cell. Then, 5 µl Annexin V-FITC (20 µg/ml) and 10 µl PI (50 µg/ml) (Beyotime, Shanghai, China) were added and cultured for 20 min in darkness at room temperature. Attune Flow Cytometer (Invitrogen, USA) was performed to measure distribution of cells. Apoptotic cells were identified as Annexin V-FITC positive and PI negative cells. The detection was repeated three times.

Statistical analysis

All data were displayed as mean±SD and analyzed using SPSS 19.0 (USA) and GraphPad Prism 7.0 (USA). Statistical significances of groups were compared with student's t-test and one way ANOVA and P<0.05 was deemed to have statistical meaning.

RESULTS

LPS induced upregulation of NMDAR subunit and complement C5 and promoted apoptosis and inflammatory cytokines of chondrocytes'

To verify involvements of NMDAR and complement C5 in the progression of OA, RNA expressions of NR1, NR2A and complement C5 were measured in different concentrations of LPS induced SW1353 cells. Results showed that NR1, NR2A and C5 RNA levels were all significantly upregulated through LPS induction in a dose dependent manner (Figure 1A). Thereafter, apoptosis of SW1353 under LPS treatment was detected, indicating that growing LPS concentrations resulting in upregulated apoptosis rate of SW1353 cells (Figure 1B). Meanwhile, RNA levels of inflammatory cytokines, TNF-α and IL-6 were checked, revealing that both TNF-α and IL-6 RNA expressions were promoted in a dose dependent manner (Figure 1C). Later, NLRP3 RNA expression was examined in LPS induced SW1353 cells, showing that NLRP3 was also upregulated under LPS treatment (Figure 1D).

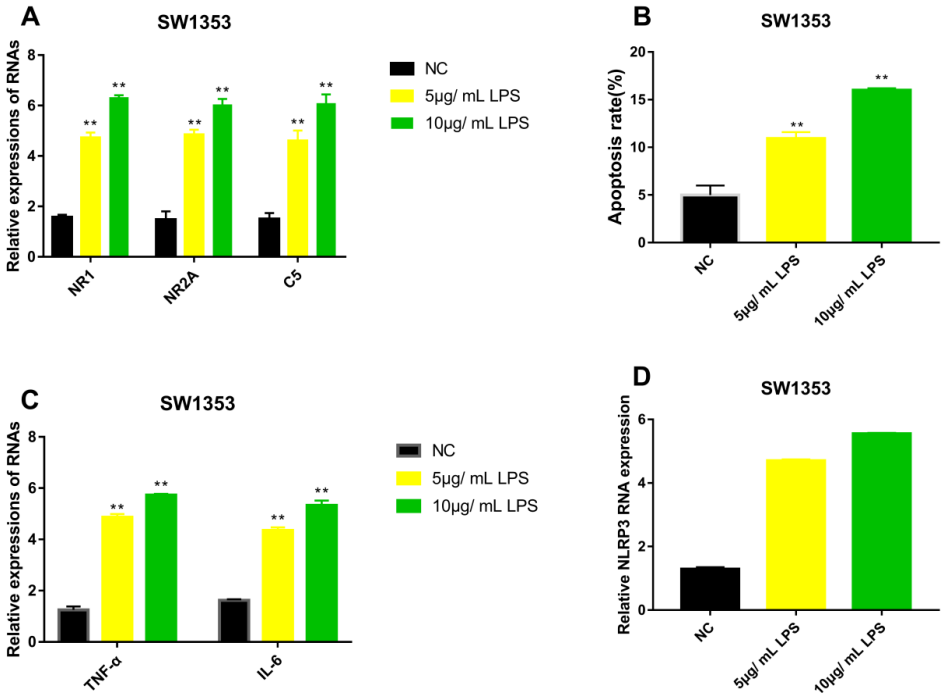

Figure 1: LPS induced upregulation of NMDAR subunit and complement C5 and promoted apoptosis and inflammatory cytokines of chondrocyte A: RNA expressions of NR1, NR2A and C5 were evaluated by RT-qPCR in SW1353 cells under different concentrations of LPS (5µg/ml and 10µg/ml), P<0.05. B: Apoptosis rate of SW1353 cells treated with different densities of LPS (5µg/ml and 10µg/ml) were analyzed by flow cytometry, P<0.05. C: TNF-α and IL-6 RNA expressions in LPS (5µg/ml and 10µg/ml) treated SW1353 cells were assessed with RT-qPCR, P<0.05. D: Relative NLRP3 RNA expressions were validated by RT-qPCR in LPS (5µg/ml and 10µg/ml) treated SW1353 cells, P<0.05

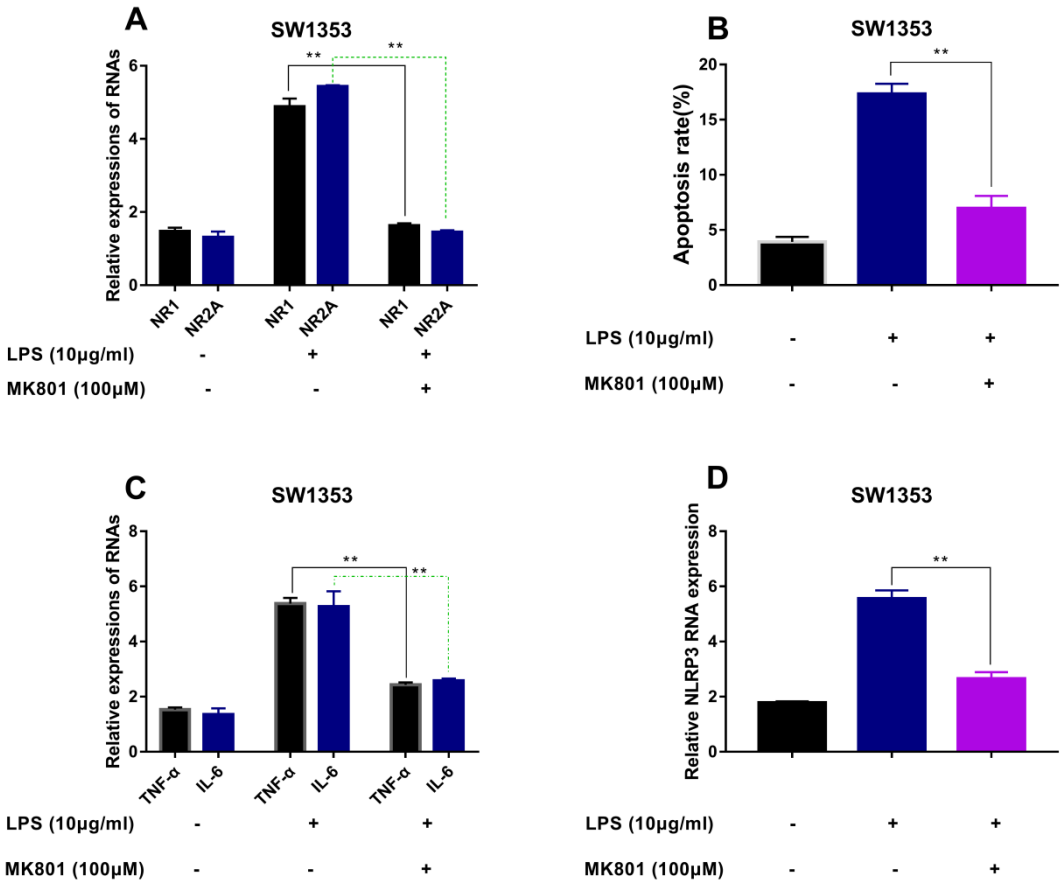

Figure 2: NMDAR antagonist attenuated LPS induced NMDAR subunit and released apoptosis and inflammation in SW1353 cells A: RNA expressions of NR1 and NR2A treated with LPS (10 μg/ml) and MK801 (100μM) were assessed by RT-qPCR in SW1353 cells, P<0.05. B: Apoptosis rate of SW1353 cells under LPS (10μg/ml) and MK801 (100 μM) treatment were checked by flow cytometry, P<0.05. C: TNF-α and IL-6 RNA levels were detected using RT-qPCR in LPS (10μg/ml) and MK801 (100 μM) treated SW1353 cells, P<0.05. D: Relative NLRP3 RNA expression in SW1353 cells after LPS (10μg/ml) and MK801 (100μM) treatment, P<0.05.

NMDAR antagonist attenuated LPS induced NMDAR subunit and released apoptosis and inflammation in SW1353 cells

After RNA expressions of NR1 and NR2A were analyzed in 10μg/ml LPS induced SW1353 cells, mediation of NMDAR antagonist, MK801 (100μM) was measured. After MK801 treatment, both NR1 and NR2A RNA expressions were significantly downregulated compared to untreated SW1353 cells (Figure 2A). Besides that, the apoptosis rate of LPS induced SW1353 cells was markedly reduced after MK801 treatment (Figure 2B). Detections of TNF-α and IL-6 RNA expressions also indicated that added MK801 obviously reduced LPS caused high RNA levels of TNF-α and IL-6 (Figure 2C). Later, MK801 treatment in mediating NLRP3 was detected, indicating that LPS induced upregulated NLRP3 was significantly reversed to low level by MK801 (Figure 2D).

Complement C5 declined LPS induced NMDAR subunit and apoptosis and inflammation of SW1353 cells

After functions of NMDAR in SW1353 cells were examined, role of complement C5 was checked. According to previous experiment, C5 was highly expressed in LPS induced SW1353 cells. Therefore, we used eculizumab, a C5 inhibitor, to figure out mechanism of C5 with NMDAR in progression of OA. As eculizumab (100 μg/ml) applied, LPS caused high expression of C5 was significantly declined (Figure 3A). Thereafter, eculizumab and MK801 treatment resulted in lower apoptosis rate of SW1353 cells compared to SW1353 cells treated by MK801 only (Figure 3B). Following, TNF-α and IL-6 RNA expressions were remarkably decreased under MK801 and eculizumab treatment in comparison with MK801 treatment (Figure 3C). Furthermore, NLRP3 RNA expression was dramatically downregulated after treated by MK801 and added eculizumab leaded to a much lower level of NLRP3 (Figure 3D).

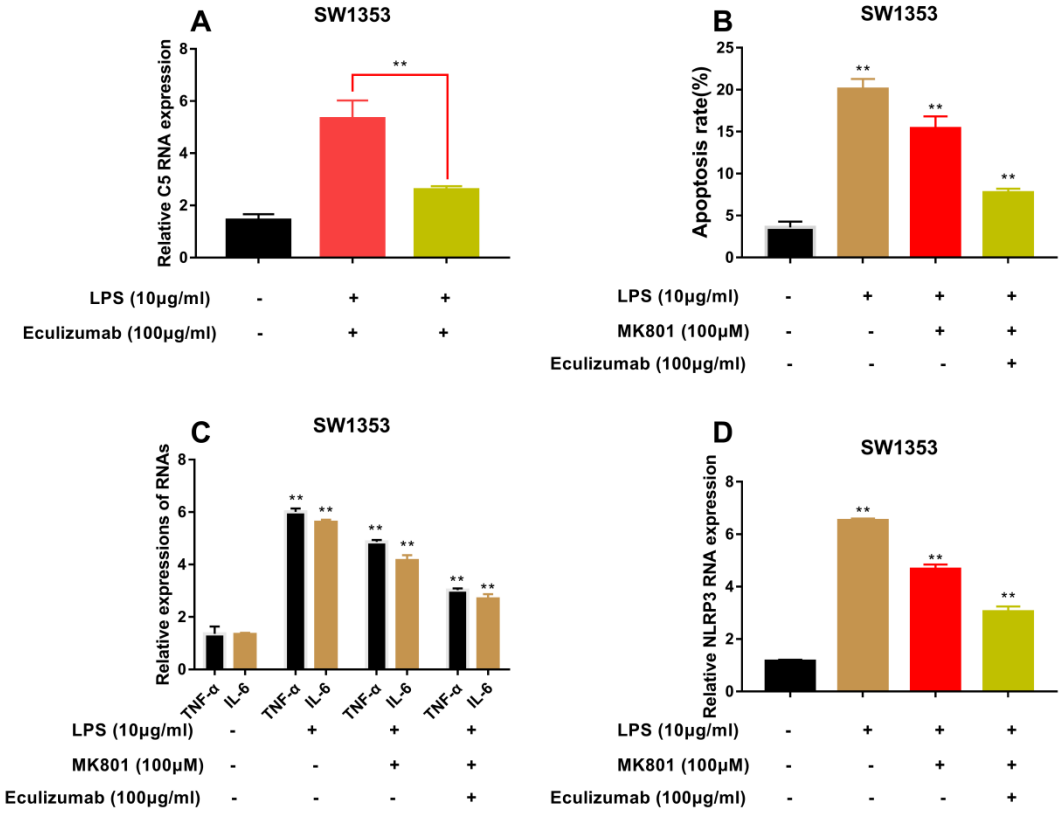

Figure 3: Complement C5 declined LPS induced NMDAR subunit and apoptosis and inflammation of SW1353 cells A: C5 RNA expression in SW1353 cells with LPS (10 μg/ml) and Eculizumab (100 μg/ml) were measured by RT-qPCR, P<0.05. B: Apoptosis rate of SW1353 cells under LPS (10μg/ml), MK801 (100 μM) and Eculizumab (100 μg/ml) were detected by flow cytometry, P<0.05. C: TNF-α and IL-6 RNA expressions were evaluated by RT-qPCR in SW1353 cells with LPS (10μg/ml), MK801 (100 μM) and Eculizumab (100 μg/ml) treatment, P<0.05. D: NLRP3 RNA level in LPS (10 μg/ml), MK801 (100 μM) and Eculizumab (100μg/ml) treated SW1353 cells were analyzed by RT-qPCR, P<0.05

DISCUSSION

According to previous studies, NMDAR worked as a pro-inflammatory factor has been deeply analyzed [30,31]. Normally, NMDAR subunits were represented as objects in progression of OA and suppression of those subunit could retard inflammation in OA [30]. Correspondingly, we also measured functions of NMDAR in SW1353 cells. At the beginning, NR1, NR2A and C5 were apparently upregulated in SW1353 cells after treated by LPS (5 μg/ml and 10 μg/ml) in a does dependent manner. Moreover, the apoptosis rate of SW1353 was also increased in a dose dependent manner. Furthermore, growing densities of LPS induced upregulation of TNF-α and IL-6 in SW1353 cells were markedly upregulated. Beyond these, NMDA was reported to induce NLRP3 through mediating retinal excitotoxicity in research of Pavlina Tsoka et al. [32]. NLRP3 is the one of the most studied inflammasomes which can activate caspase-1 and resulting in secretions of inflammatory cytokines IL-1β, IL-18 and TNF-α [33]. In previous studies, inhibition of NLRP3 leaded to suppression of pro-inflammatory response in OA samples and cells [34]. Moreover, Icariin attenuated LPS induced inflammation and apoptosis by repressing NLRP3 signaling [35]. NMDA was proven to promote activation of NLRP3 and its activation to caspase-1 needed to be stimulated by activation of C5[32, 36]. Based on these detections, we measured NLRP3 as a biomarker in progression of OA. In out study, NLRP3 RNA expression. Results indicated that LPS simulated OA inflammation could accelerate expressions of NMDAR and complement C5 and upregulated NLRP3 expression. According to our detections, we primarily knew that in LPS induced SW1353 cells, subunits of NMDAR, C5 and NLRP3 have the same tendency.

After that, NMDAR antagonist, MK801 was used to analyze role of NMDAR in LPS induced SW1353 cells. In RNA expressions of NR1 and NR2A detection, MD801 treatment (100 μM) significantly reduced RNA levels of these two subunits which were promoted by LPS (10 μg/ml). Besides that, upregulated LPS induced apoptosis rate of SW1353 cells was dramatically

reduced under MK801 treatment. Furthermore, TNF-α, IL-6 and NLRP3 RNA expression were also declined with MK801 treatment after promoted by LPS. Through these detections, blocking of NMDAR was determined to retard inflammation and apoptosis of SW1353 cells through downregulating subunits NR1 and NR2A. Compared to former studies, we have measured a new way about MK801 suppressing inflammation of OA.

Giuseppina Arbore et al. have declared that assembly of NLRP3 required activation of C5 [36]. Therefore, we measured suppression of C5 in OA to dig out regulation of C5 to NLRP3 in OA. As involvement of NMDAR was examined, role of complement C5 was measured later. Eculizumab was first performed to suppress C5 RNA expression and result of RT-qPCR showed that RNA level of C5 was reversed to low expression in eculizumab (100 μg/ml) treated LPS-SW1353 cells. Thereafter, correlation between MK801 and eculizumab was checked later, revealing that eculizumab treatment enhanced suppression of apoptosis caused by MK801. Moreover, TNF-α, IL-6 and NLRP3 RNA expressions in LPS induced SW1353 cells with MK801 were also declined after eculizumab treatment. Therefore, C5 played identical functions in facilitating inflammation and apoptosis of SW1353 with NMDAR and eculizumab blocked its role with suppressing NLRP3 and other inflammatory cytokines. Compared to other studies, we have analyzed correlation of C5 and NMDAR in progression of OA and their mediations to NLRP3 inflammasome.

CONCLUSION

NMDAR subunit NR1 and NR2A and complement C5 were all upregulated in LPS induced SW1353 cells and promoted apoptosis and TNF-α, IL-6 and NLRP3 expressions while MK801 inhibited functions of NMDAR and eculizumab suppressed role of complement C5 to retard inflammation and apoptosis of SW1353 cells, indicating that NMDAR and complement C5 could be potential biomarkers to examine inflammatory progression of OA. However, Further studies in vivo and in clinical stage are requested for getting more knowledge of those two factors.

DECLARATIONS

List of Abbreviations

NMDAR: N-methyl-D-aspartate receptor; NLRP3: nucleotide-binding oligomerization domain-, leucine-rich repeat- and pyrin domain-containing 3; OA: osteoarthritis; TNF-α: Tumor Necrosis Factor-α; IL-6: interleukin 6 NR1: N-methyl-d-aspartate receptor 1; NR2A: N-methyl-d-aspartate receptor 2A; C5: complement C5.

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Loeser RF, Goldring SR, Scanzello CR,Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis and rheumatism 2012; 64(6): 1697-1707.*
2. *Blagojevic M, Jinks C, Jeffery A,Jordan KP. Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. Osteoarthritis*

and cartilage 2010; 18(1): 24-33.
3. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, Kington RS, Lane NE, Nevitt MC, Zhang Y et al. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Annals of internal medicine 2000; 133(8): 635-646.
4. Woolf AD,Pfleger B. Burden of major musculoskeletal conditions. Bulletin of the World Health Organization 2003; 81(9): 646-656.
5. Bijlsma JW, Berenbaum F,Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet (London, England) 2011; 377(9783): 2115-2126.
6. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis and cartilage 2013; 21(1): 16-21.
7. Goldring MB,Otero M. Inflammation in osteoarthritis. Current opinion in rheumatology 2011; 23(5): 471-478.
8. Zhe Z, Hongyuan B, Wenjuan Q, Peng W, Xiaowei L,Yan G. Blockade of glutamate receptor ameliorates lipopolysaccharide-induced sepsis through regulation of neuropeptides. Bioscience reports 2018; 38(3).
9. Liu Y,Zhang J. Recent development in NMDA receptors. Chinese medical journal 2000; 113(10): 948-956.
10. Minnella AM, Zhao JX, Jiang X, Jakobsen E, Lu F, Wu L, El-Benna J, Gray JA,Swanson RA. Excitotoxic superoxide production and neuronal death require both ionotropic and non-ionotropic NMDA receptor signaling. Scientific reports 2018; 8(1): 17522.
11. Leung JC, Travis BR, Verlander JW, Sandhu SK, Yang SG, Zea AH, Weiner ID,Silverstein DM. Expression and developmental regulation of the NMDA receptor subunits in the kidney and cardiovascular system. American journal of physiology. Regulatory, integrative and comparative physiology 2002; 283(4): R964-971.
12. Crespi F, Lazzarini C, Andreoli M,Vecchiato E. Voltammetric and functional evidence that N-methyl-D-aspartate and substance P mediate rat vascular relaxation via nitrogen monoxide release. Neuroscience letters 2000; 287(3): 219-222.
13. Wu QJ,Tymianski M. Targeting NMDA receptors in stroke: new hope in neuroprotection. Molecular brain 2018; 11(1): 15.
14. Chopra A, N-{2-[4-(4-[(125)I]Iodobenzyl)-piperidin-1-ylmethyl]benzoimidazol-5-yl}-methanesulfonamide, in Molecular Imaging and Contrast Agent Database (MICAD). 2004, National Center for Biotechnology Information (US): Bethesda (MD).
15. Piepoli T, Mennuni L, Zerbi S, Lanza M, Rovati LC,Caselli G. Glutamate signaling in chondrocytes and the potential involvement of NMDA receptors in cell proliferation and inflammatory gene expression. Osteoarthritis and cartilage 2009; 17(8): 1076-1083.
16. Neher MD, Weckbach S, Flierl MA, Huber-Lang MS,Stahel PF. Molecular mechanisms of inflammation and tissue injury after major trauma--is complement the "bad guy"? Journal of biomedical science 2011; 18(1): 90.
17. Carroll MV,Sim RB. Complement in health and disease. Advanced drug delivery reviews 2011; 63(12): 965-975.
18. Thomas AM, Gerogianni A, McAdam MB, Fløisand Y, Lau C, Espevik T, Nilsson PH, Mollnes TE,Barratt-Due A. Complement Component C5 and TLR Molecule CD14 Mediate Heme-Induced Thromboinflammation in Human Blood. Journal of immunology (Baltimore, Md. : 1950) 2019; 203(6): 1571-1578.
19. Ward PA. The harmful role of c5a on innate immunity in sepsis. Journal of innate immunity 2010; 2(5): 439-445.
20. Lusthaus M, Mazkereth N, Donin N,Fishelson Z. Receptor-Interacting Protein Kinases 1 and 3, and Mixed Lineage Kinase Domain-Like Protein Are Activated by Sublytic Complement and Participate in Complement-Dependent Cytotoxicity. Frontiers in immunology 2018; 9: 306.
21. Vicenti G, Bizzoca D, Carrozzo M, Solarino G,Moretti B. Multi-omics analysis of synovial fluid: a promising approach in the study of osteoarthritis. Journal of biological regulators and homeostatic agents 2018; 32(6 Suppl. 1): 9-13.
22. Dragomir CL, Scott JL, Perino G, Adler R, Fealy S,Goldring MB. Acute inflammation with induction of anaphylatoxin C5a and terminal complement complex C5b-9 associated with multiple intra-articular injections of hylan G-F 20: a case report. Osteoarthritis and cartilage 2012; 20(7): 791-795.
23. Wehling C, Amon O, Bommer M, Hoppe B, Kentouche K, Schalk G, Weimer R, Wiesener M, Hohenstein B, Tönshoff B et al. Monitoring of complement activation biomarkers and eculizumab in complement-mediated renal disorders. Clinical and experimental immunology 2017; 187(2): 304-315.
24. Chen L, Li Q, Wang J, Jin S, Zheng H, Lin J, He F, Zhang H, Ma S, Mei J et al. MiR-29b-3p promotes chondrocyte apoptosis and facilitates the occurrence and development of osteoarthritis by targeting PGRN. Journal of cellular and molecular medicine 2017; 21(12): 3347-3359.
25. Li F, Sun J, Huang S, Su G,Pi G. LncRNA GAS5 Overexpression Reverses LPS-Induced Inflammatory Injury and Apoptosis Through Up-Regulating KLF2 Expression in ATDC5 Chondrocytes. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 2018; 45(3): 1241-1251.
26. Chen Z, Wang X, Ashraf U, Zheng B, Ye J, Zhou D, Zhang H, Song Y, Chen H, Zhao S et al. Activation of neuronal N-methyl-D-aspartate receptor plays a pivotal role in Japanese encephalitis virus-induced neuronal cell damage. Journal of neuroinflammation 2018; 15(1): 238.
27. Xu D, Yi H, Yu S, Li X, Qiao Y,Deng W. Association of Complement C5 Gene Polymorphisms with Proliferative Diabetic Retinopathy of Type 2 Diabetes in a Chinese Han Population. PloS one 2016; 11(3): e0149704.
28. Ridge JP, Ho AM, Innes DJ,Dodd PR. The expression of NMDA receptor subunit mRNA in human chronic alcoholics. Annals of the New York Academy of Sciences 2008; 1139: 10-19.
29. Tian R, Zhu Y, Yao J, Meng X, Wang J, Xie H,Wang R. NLRP3 participates in the regulation of EMT in bleomycin-induced pulmonary fibrosis. Experimental cell research 2017; 357(2): 328-334.
30. Salter DM, Wright MO,Millward-Sadler SJ. NMDA receptor expression and roles in human articular chondrocyte mechanotransduction. Biorheology 2004; 41(3-4): 273-281.
31. Ramage L, Martel MA, Hardingham GE,Salter DM. NMDA receptor expression and activity in osteoarthritic human articular chondrocytes. Osteoarthritis and cartilage 2008; 16(12): 1576-1584.
32. Tsoka P, Barbisan PR, Kataoka K, Chen XN, Tian B, Bouzika P, Miller JW, Paschalis EI,Vavvas DG. NLRP3 inflammasome in NMDA-induced retinal excitotoxicity. Experimental eye research 2019; 181: 136-144.
33. Place DE,Kanneganti TD. Recent advances in inflammasome biology. Current opinion in immunology 2018; 50: 32-38.
34. Chen Z, Zhong H, Wei J, Lin S, Zong Z, Gong F, Huang X, Sun J, Li P, Lin H et al. Inhibition of Nrf2/HO-1 signaling leads to increased activation of the NLRP3 inflammasome in osteoarthritis. Arthritis research & therapy 2019; 21(1): 300.
35. Zu Y, Mu Y, Li Q, Zhang ST,Yan HJ. Icariin alleviates osteoarthritis by inhibiting NLRP3-mediated pyroptosis. Journal of orthopaedic surgery and research 2019; 14(1): 307.
36. Arbore G, West EE, Spolski R, Robertson AAB, Klos A, Rheinheimer C, Dutow P, Woodruff TM, Yu ZX, O'Neill LA et al. T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in CD4$^+$ T cells.

Science (New York, N.Y.) 2016; 352(6292): aad1210..