Hypoxia Induced factor2αEffects of estrogen on Rats

Condylar Cartilage Cells Effect of Study
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Abstract: Objective: Investigate the hypoxia induced factor2α(HIF2α) In estrogen stimulation under the mandibular condylar cartilage cells effect in role.
KeyWords: estrogen; hypoxia induced factor; temporal mandibular joint; condylar; cartilage cells

Is a key factor mediating the low oxygen effect..In recent years, studies have found that,In osteoarthritis (Osteoarthritis,OA)Cartilage TissueHif1Alpha,[4.-6.],Hif2AlphaThe expression levelAnd knockoutHif2Alpha[4-5.]Gene can effectively reduce the severity of knee inflammation.However, the relationship between Estrogen andHIFInTMDOf the development whether there interaction effect has not been See related reports?.This study investigateHIF2αIn the effect of estrogen on mandibular condylar cartilage cells in specific role mechanism in order toTMDOf clinical treatment provide theory basis.

1. Material and Methods

1.1 Main reagent and Instrument

Fetal bovine serum (Hyclone SH30084.03),High GlucoseDMEMCell medium (Hyclone SH30022.01)(Australia blood Australia );Trypsin (T7309),Dimethyl sulfoxide (DMSOD2650)(SigmaAmerican);Style collagenase(Col)(17101-015)(InvitrogenAmerican);17β-Estradiol(120657),HIF2αAntibody (Ab8365),AggrecanAntibody(3778),MMP13Antibody (39012),XType collagen (Col)Antibody (Ab58632)(AbcamAmerican);Estrogen Receptor BlockerICI 182780(S1191)(SelleckchemAmerican);HIF2αInhibitor(CAS 882268-69-1)(MerckGermany);

1.2 Condylar Cartilage Cells of in vitro culture and Identification

Sterile Separation3Weeks ageSprague-Dawley(SD)RatsThe Mandibular Condyle of the Fourth Military Medical University was harvested from the central part of the translucent cartilage and cut into a sterile petri dish.1mm x 1mmPieces of size;Join2..5g/L.Trypsin37Digestion1 HAfter,1 000 r/minCentrifugal5 min,Discard the supernatant;Joinagain1g/L Col 37Continue to digest2 h;End digestion200Mesh Filter and collect cells by centrifugation;With containing200/L.Fetal bovine serumDMEMThe cells were made into suspension in the medium, and the cells were purified by repeated Adherent Method and mechanical curettage method, respectively..To simulate condylar chondrocytes in vivo.In the hypoxic environment, the cells50 mL/L O2.
Take the thriving Section 2. The phenotype of chondrocytes was identified by immunohistochemical staining of collagen type. Specific steps: routine production of cell climbing tablets, 40 g/L paraformaldehyde fixation 30 min, and PBS cleaning 5 min x 3. After the second time, and then contain 1 g/L Triton-OI PBS dissolve, liquid, soak 15 min; PBS rinsing, wash 5 min x 3. Second, add cell closing solution, and seal at room temperature 30 min; Primary antibody to collagen (1:100), 4. Incubate overnight; next day PBS cleaning 3 times, drops plus two second antibody, incubated at room temperature 30 min;
PBS cleaning 3 times, DAB color; Hematoxylin staining 3 min. After conventional dehydration, transparent, neutral gum seal, observe and take pictures under the microscope.

1.3 Cell grouping and Intervention

Will no 2. Cartilage Cells of the condyle 2x10⁶/Density of dish inoculated in diameter 100 mm. Add a petri dish containing 100 mL fetal bovine serum DMEM culture medium, until cell growth converge to approximately 85%. Discard the original culture medium and divide the cells A, B, C, D 4 Group (N = 3). A Group for estrogen stimulation (E2) Group, join 1 μM/L 17β-Estradiol DMEM Medium; B Group for estrogen stimulation + Estrogen Receptor Blockade (E2 + ICI 182780) Group,

Inham1 μM/L 17β-Estradiol, 1 μM/L 2H; 182780 of DMEM Medium; C Group for estrogen stimulation Hif2α Blocking (E2 hif2α Inhibitor) Group, join μM/L 17β-Estradiol, 5 μM/L hif2α Inhibitor DMEM Culture medium; D Group for control group adding an equal amount

DMEM Culture Medium. Continue to training 12 h. After collection each group cells (Each sample about 1 × 10⁷ A cells) For follow-up Detection.

1.4 Real-time quantitative PCR Detection

60 Degeneration 34 S Repeat 40 A cycle; finally again 95 Degeneration 15 S 60 Degeneration 60 s 95 Degeneration 15 s. The primers by day

Takara The company Design and Synthesis specific primers sequence see table 1. Anti-Should be. After application system comes with Analysis Software CFX Manager 3.1 2-CT.

Analysis data Method Calculation groups gene of Expression Level

1.5 Western blot Detection

Extraction 1.3 In each group cells of total protein BCA Protein Quantitative Detection Kit determination protein concentration; with twelve sodium dodecyl sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) The protein separation and Will protein go PVDF Membrane; Then 50 g/L skim milk powder closed after respectively join Aggrecan (1:500), HIF2α 1:400), MMP13 (1:500), VEGF (1:800), Col X 1:500), β-Actin (1:1 000), A 4 Refrigerator incubation night; The VALUES Wash the membrane after join two anti-incubated at room temperature 1 H; Again

The VALUES Wash the membrane ECL Fa guang ye color take pictures.

1.6 Statistical Analysis

SPSS 18.0 Software of data the single factors variance analysis (One-Way anova) Two compare the T Test test water

2. Results

2.1 Condylar Cartilage Cells of training and Identification

The original s Condylar Cartilage Cells 5 DA about covered the bottom of the bottle microscope under visible cells form was paving stones-like for Polygon, Star (Figure 1A). This experimental select of the first 2S condylar cartilage cells the style collagen immune was staining results display cells staining were positive (Figure 1B).
2.2 Each group cartilage cells the matrix and pro-inflammatory factor mRNA Change of expression

As shown in Fig.2. As shown, compared with the control group, A Group and C Group cartilage cells Aggrecan, Col of mRNA The expression level was significantly reduced (P<0.05); Simultaneous A Group chondrocyte-associated proinflammatory factors II-1 Beta, TNF-Alpha of mRNA Up-regulated expression (P<0.05). With A Group comparison, B Group and C Group cartilage cells Aggrecan, Col of mRNA Up-regulated expression (P<0.05) Cartilage-related proinflammatory factors II-1 Beta, TNF-Alpha mRNA The expression level was significantly reduced (P<0.05). It suggests that estrogen receptor blockers ICI 182780 And Hif2Alpha Blocking Agents can reverse the expression of extracellular matrix and pro-inflammatory factors in Chondrocytes stimulated by estrogen.

2.3 Hif2Alpha And its downstream Factors mRNA Expression Change Mmp13, VEGF, Col of mRNA Significant expression

As shown in Fig.3. As shown, compared with the control group, A Group cartilage cells Tune (P<0.05). Suggest that estrogen receptor blockers and Hif2Alpha Hif2Alpha And its downstream factor Mmp13, VEGF, Col of MR Blocking Agents can inhibit estrogen-induced Hif2Alpha And below mRNA The expression level was significantly up-regulated (P<0.05). With A Group comparison, Expression level of swim factor.

B Group and C Group cartilage cells Hif2Alpha And its downstream factor

2.4 Aggrecan, Hif2Alpha Expression changes of Protein and Its Downstream Factors

As shown in Fig.4.-5. As shown, compared with the control group, A Group cartilage cells Aggrecan The protein expression (P<0.05), And Hif2Alpha And its downstream factor Mmp13, VEGF And Col x The egg

3. Discussion

TMD Refers to the temporomandibular joint (TMJ) And/or a common oral disease of the masticatory muscles. 20%~7.40% The main clinical manifestations are maxillofacial pain, Joint Noise Wait. In vitro culture of mandibular condylar Chondrocytes

High concentration (10^-6 Mol/L) Estrogen can inhibit the proliferation of chondrocytes and the synthesis of proteoglycan. Our previous study also confirmed that excessive genistein (A phytoestrogen) could induce Col, Aggrecan And proliferating cell nuclear antigen (PCNA) Significantly down-regulated the expression

The stimulation of estrogen can Aggrecan And Col of mRNA Expression levels were significantly reduced, while promoting Inflammatory Factors II-1, TNF-Alpha And matrix degradation factor Mmp13 Of MRNA Up-regulated expression. All the above results suggest that high concentrations of estrogen can induce the cartilage of condylar chondrocyte

Other studies show that, ERAlpha It participates in the Proliferation Effect of hypoxia on breast cancer stem cells. ERAlpha Antagonist Resistance

Collaboration relationship. The results showed that high concentrations of estrogen could significantly up-regulate the degeneration of condylar chondrocytes. Hif2Alpha And its downstream factor Mmp13, VEGF, Col Protein Expression Level. The results are consistent with previous reports OA Cartilage Tissue Hif2Alpha[4.-5.].

The results further confirmed that high concentrations of estrogen could be directly up-regulated. Hif2Alpha Up-regulation of its downstream factor expression can accelerate the process of chondrocyte degeneration. We also found that estrogen receptor blockers and Hif2Alpha Blocking Agents can reverse estrogen-induced Hif2Alpha Up-regulation of downstream Factor Expression, Cartilage Matrix Synthesis reduction and
other effects. According to the analysis, estrogen-Estrogen Receptor-Hif2AlphaThe presence of pathway may be in condylar cartilage OA plays an important role in the occurrence and development.

References