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ORIGINAL ARTICLE

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(Ab120657),HIF2αAntibody (Ab8365),AggrecanAntibody(Ab3778),MMP13Antibody (Ab39012),XType collagen (Col)Antibody (Ab58632)(AbcamAmerican);Estrogen Receptor BlockerICI 182780(S1191)(SelleckchemAmerican);HIF2αInhibitor(CAS 882268-69-1)(MerckGermany);RNAReverse KitPrime ScriptRT Master Mix(RR036A), REAL-Time PCRReaction kitSYBRPremix Ex Taq™(RR820A)(TakaraJapanese);VEGFAntibody (19003-1-AP)(ProteintechAmerican);BCAProtein quantitative Kit(23227),ECLChemiluminescent(32109)(PierceAmerican);5 × SDS-PAGEProtein on-like buffer (Shanghai, Pik Wan days); optical microscope (cuisine card Germany).

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.Mm x 1mm x 1mmPieces of size;Join2..5g/LTrypsin37Digestion1 HAfter,1 000 r/minCentrifugal5 min,Discard the supernatant;Join again1g/L Col 37Continue to digest2 h;End digestion200Mesh Filter and collect cells by centrifugation;With containing200/LFetal 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 O_2.,

Take the thriving Section2.The phenotype of chondrocytes was identified by immunohistochemical staining of collagen type..Specific steps: routine production of cell climbing tablets,40g/LParaformaldehyde Fixation30 min, AndPBSCleaning5 min x 3After the second time, and then contain1g/L TritonOfPBSDissolve, liquid, soak15 min;PBSQing, wash 5 Min x 3Second, add cell closing solution, and seal at room temperature.30 min;Primary antibody to collagen (1 100),4.Incubate overnight; next dayPBSCleaning3.Times, drops plus two second antibody, incubated at room temperature30 min;

PBSCleaning3.Time,DabColor;Hematoxylin Staining3 minAfter conventional dehydration,Transparent, neutral gum seal, observe and take pictures under the microscope.

1.3 Cell grouping and Intervention

Will no2.Cartilage Cells of the condyle2x10^{6}/Density of dish inoculated in diameter100Add a petri dish containing100/LFetal bovine serumDMEMCulture Medium, until cell growth converge to approximately85%Discard the original culture medium and divide the cellsA,B,C,D 4 Group (N = 3).AGroup for estrogen stimulation (E2)Group, join 1 μMol/L 17Beta-EstradiolDMEMMedium;BGroup for estrogen stimulation+Estrogen Receptor Blockade (E2 + ICI 182780)Group,

Inham1.μMol/L 17Beta-Estradiol,1.μMol/l ici 182780OfDMEMMedium;CGroup for estrogen stimulationHif2AlphaBlocking(E2 hif2AlphaInhibitor)Group, join μMol/L 17Beta-Estradiol,5.μMol/L hif2AlphaInhibitorDMEMCulture medium;DGroup for control group adding an equal amount

DMEMCulture Medium.Continue to training12 hAfter collection each group cells(Each sample about1 × 10^{7}/A cells)For follow-up Detection.

1.4 Real-time quantitativePCRDetection

60Degeneration34 SRepeat40A cycle; finally again95Degeneration 15 S60Degeneration60 s95Degeneration15 s.The primers by day

TakaraThe company Design and Synthesis specific primers sequence see table1.Anti-Should be. After application system comes with Analysis SoftwareCFX Manager 3.1 2- CT .

Analysis data Method Calculation groups gene of Expression Level

1.5 Western blotDetection

Extraction1.3In each group cells of total proteinBCAProtein

Quantitative Detection Kit determination protein concentration; with twelve sodium dodecyl sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE)The protein separation and Will protein goPVDFMembrane;Then50g/LSkim milk powder closed after respectively joinAggrecan(1:500),HIF2α

1:400),MMP13(1:500),VEGF(1:800),Col X

1:500),β-Actin(1:1 000)A,4Refrigerator incubation night;The VALUESWash the membrane after join two anti-incubated at room temperature1 H;Again

The VALUESWash the membraneECLFa guang ye color take pictures.

1.6 Statistical Analysis

SPSS 18.0Software of data the single factors variance analysis (One-Way anvoa)Two compare theTTest test water

2. Results

2.1 Condylar Cartilage Cells of training and Identification

The original s Condylar Cartilage Cells5 dAbout covered the bottom of the bottle microscope under visible cells form was paving stones-like for Polygon,Star (Figure1a).This experimental select of the first2S condylar cartilage cells the style collagen immune was staining results display cells staining were was positive (Figure1B).

2.2 Each group cartilage cells the matrix and pro-inflammatory factorMRNAChange of expression

As shown in Fig.2.As shown, compared with the control group,AGroup andCGroup cartilage cellsAggrecan,ColOfMRNaThe expression level was significantly reduced (P<0.05);SimultaneousAGroup chondrocytes-associated proinflammatory factorsIl-1.Beta,TNF-AlphaOfMRNaUp-regulated expression(P<0.05).WithAGroup comparison,BGroup andCGroup cartilage cells

Aggrecan,ColOfMRNaUp-regulated expression

(P<0.05)Cartilage-related proinflammatory factorsIl-1.Beta,TNF-Alpha

MRNaThe expression level was significantly reduced (P<0.05).It suggests that estrogen receptor blockersICI 182780AndHif2AlphaBlocking Agents can reverse the expression of extracellular matrix and pro-inflammatory factors in Chondrocytes stimulated by estrogen·

2.3 Hif2AlphaAnd its downstream FactorsMRNaExpression ChangeMmp13,VEGF,ColOfMRNaSignificant expression

As shown in Fig.3.As shown, compared with the control group,AGroup cartilage cellsTune (P<0.05).Suggest that estrogen receptor blockers andHif2Alpha

Hif2AlphaAnd its downstream factorMmp13,VEGF,ColOfMR-Blocking Agents can inhibit estrogen-inducedHif2AlphaAnd below

NaThe expression level was significantly up-regulated (P<0.05).WithAGroup comparison,Expression level of swim factor.

BGroup andCGroup cartilage cellsHif2AlphaAnd its downstream factor

2.4 Aggrecan,Hif2AlphaExpression changes of Protein and Its Downstream Factors

As shown in Fig.4.~5.As shown, compared with the control group,AGroup cartilage cellsAggrecanThe protein expression(P<0.05), AndHif2AlphaAnd its downstream factorMmp13,VEGFAndCol xThe egg

3. Discussion

TMDRefers 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 induceCol,AggrecanAnd proliferating cell nuclear antigen (PCNA)Significantly down-regulated the expression

The stimulation of estrogen canAggrecanAndColOfMRNaExpression levels were significantly reduced, while promoting Inflammatory FactorsIl-1.,TNF-AlphaAnd matrix degradation factorMmp13OfMRNaUp-regulated expression.All the above results suggest that high concentrations of estrogen can induce the cartilage of condylar chondrocyte

Other studies show that,ERAlphaIt participates in the Proliferation Effect of hypoxia on breast cancer stem cells.ERAlphaAntagonist Resistance

Collaboration relationship.The results showed that high concentrations of estrogen could significantly up-regulate the degeneration of condylar chondrocytes.Hif2AlphaAnd its downstream factorMmp13,VEGF,ColProtein Expression Level.The results are consistent with previous reportsOACartilage TissueHif2Alpha[4.-5.],

The results further confirmed that high concentrations of estrogen could be directly up-regulated.Hif2AlphaUp-regulation of its downstream factor expression can accelerate the process of chondrocyte degeneration..We also found that estrogen receptor blockers andHif2AlphaBlocking Agents can reverse estrogen-inducedHif2AlphaUp-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 cartilageOAPlays an important role in the occurrence and development.

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