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Nile TilapiaXbp1-SGene Cloning and Expression Analysis

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Abstract: To learn about Nile tilapia(Oreochromis niloticus)Cell Transcription FactorXbp1-S(OnXbp1-S)Sequence characteristics of the Gene and Its Role in Streptococcus agalactiae(Streptomyces alactolyticus)Stress and inBRole in cell differentiation, ApplicationRaceObtained by cloning technologyOnXbp1-SFull Length of Gene1380 BPIncluding open reading frameORFFor1155 BP,5.Wang YiTerminal non-coding region(5)Wang YiUTR)

The organization in have expression,MRNALevel on in liver in expression highest protein level in thymus in expression highest and in muscle in expression minimum. No milk Streptococcus stress after,OnXbp1-SGene in liver and spleen in expression trend similar were in stress during appear expression was up-regulated in192 hThe peak. In addition immune was analysis found,OnXbp1-SFactor in different differentiation degreeBCells subclass in Expression Difference in matureBCells in present high expression and in not matureBCells in almost don't expression. Research results show that,OnXbp1-SParticipate in Nile tilapia on no milk streptococcus of immune defense and inBCells Differentiation role. This study will for further studyOnXbp1-SFactor response pathogenic bacteria infection of mechanism and promoteBCells Differentiation Mechanism provide theory basis.

Keywords: Nile tilapia;Xbp1-S; Gene cloning; Organization expression; no milk streptococcus

Xbp1-SIs a highly conservative protein from low yeast to Higher Nursing animal in there(LiouEt al1990). In the bony fish,Xbp1-SThe Research of fish, including zebrafish, is gradually increasing.(Dario rerio) (HuEt al, 2007; BennettEt al, 2007)Carp(Cyprinus carpio)(Peng shaoqing and others, 2013)Takifugu with red fin(Takifugu rubripes) (Ohtani)Et al, 2006)Rainbow trout(Oncorhynchus mykiss) (BarrEt al, 2011)And Atlantic salmon(Salmo salarLeongEt al, 2010)Etc.HuWait.(2007)In zebrafish embryonic cells, whenUPRWhen dealing with endoplasmic reticulum stress,XBP1Can passIGF1/AktActivation of apoptosis signal plays an inhibitory role;BarrWait.(2011)UtilizationXBP1Identification of different degree of differentiation as a markerBCell subclass;ZwolloWait.(2005,2008)UtilizationXBP1AndPax-5And other Transcription Factors on the kidney of rainbow trout, and further revealBDevelopment and differentiation of cellsXBP1Expression is related. But about Nile tilapiaXbp1-SResearch has not been reported. In addition, compared with mammals

Xbp1-SThe role of the host in response to pathogen infection remains to be further clarified.

Tilapia is one of the high-quality aquaculture varieties recommended by the United Nations. China is the largest tilapia producer. In recent years, large-scale breeding, high-density breeding, water pollution and so on have led to a large outbreak of tilapia fish disease, such as streptococcus, causing huge economic losses.

(Rumai new, 2010). In this study, Nile tilapia was selected as the experimental fish.Xbp1-SGeneCDNAGene and

protein levels were used to analyze the spatio-temporal expression patterns of tilapia tissues after Streptococcus agalactiae stress.BThe expression level of cell subclasses in Nile tilapiaXbp1-SRole and regulation in the prevention of streptococcus infectionBTo provide a theoretical basis for the mechanism of cell development and differentiation.

1. Materials and Methods

1.1 Experimental materials

Experimental fish were purchased from Guangdong fishery germplasm protection base, healthy Nile

The weight of tilapia is(200 ± 10) g, Body length(13 ± 2) cm. Breeding TemperatureDu is(29 ± 1)Feed, air pump oxygen supply, feeding14 dMake it fully adapt to the breeding environment, observe the fish body appearance and activity ability, and confirm that it is completely healthy for the experiment. Streptococcus agalactiae strain as this experiment

Room preservation strains.PCRReagents for reaction systems,PMD-18T Vector, Restriction enzymes,TrizolReverse Transcription Kit(Primescript™RT agent kit with gdna eraser)And Fluorescence Quantitative Kit(SYBR premix exTaq^{TM})Both purchased fromTakaraCompany;E. coliDH5AlphaReceptive,E. coliBL21 (DE3)Receptive,Gel recovery kit and plasmid extraction kit(Tianprep mini plasmid kit)Purchased fromTiangenCompany;PET32a () vectorPreserved by our laboratory,Smart^{TM}RACE cDNA Amplification KitPurchased fromBD Biosciences clontechCompany.Trizol,2.Wang YiPrime mix,ExTaq,10Wang YiBuffer,DNTP,PMD 18-T Vector,Solution IAnd reverse transcription kit purchase

TakaraCompany. Ampicillin, chloroform, isopropanol and other reagents were purchased from Guangzhou Chemical Reagent Company, and the rest of the experimental reagents were made in China. Primer(Table1)It was synthesized by Huada Gene Technology Service Co., Ltd.

1.2 Experimental Method

1.2.1 GeneralRNAExtraction andCDNASynthesis of the first chainAdoptedTrizol (Invitrogen)Law GeneralRNAExtraction. Take the healthy Nile tilapia head kidney, fully ground with a homogenizer, and followTrizolReagent manual extraction totalRNA,RNAIntegrity and purity1%Agarose gel electrophoresis and micro SpectrophotometerNano2000Detect its concentration,Wang Yi80C. Preservation. Reverse Transcription synthesis using a common reverse transcription KitCDNATemplate, usingSmart^{TM}RACE cDNA

Amplification kitWill totalRNAReverse Transcription5.Wang Yi,3.Wang Yi-RACE cDNATemplate(YaoEt al, 2016). After reverse transcription, the validity of the template was testedBeta-ActinAs an internal reference GenePCRAfter Qualification-20C. Preservation.

1.2.2 Nile tilapiaXbp1-SFull Length of GeneCDNACloning

AND SEQUENCINGInNCBISearch for Nile tilapiaXBP1Genes, based on the predicted Sequence(Xm_013276886.1), Application SoftwarePrimer Premier 5.0Carry PrimerXbp1-F1AndXbp1-R1Design(Table1)To Nile tilapia head kidneyCDNAAs a templateXbp1-F1AndXbp1-R1For primerXbp1-SOfCDNAThe amplification,PCRThe reaction system is25μL, Reaction procedure:94Pre-denaturation3 min;94Denaturation30 s;58.0Annealing30 s;72C Extension1.0 min;

A loop. AmplifiedPCRProduct Sutra1%After agarose gel electrophoresis, it was sent to Beijing Liuhe Hua big Gene company for sequencing. Sequencing

ResultsNCBI (http:/www. ncbi.nlm.nih.gov)Compare and get Nile tilapiaXbp1-SOfCDNASequence.

1.2.3 Nile tilapiaXbp1-SGene3.Wang YiAnd5.Wang Yi-RaceAmplification

According to the obtainedXbp1-SOfCDsSequence, designXbp1-S

Of5.Wang Yi-RACE PCRAnd3.Wang Yi-RACE PCRPrimerXbp1-F2,Xbp1-F3,Xbp1-R2AndThe Xbp1-R3 (Table1), Using nestedPCR

Method proceed3.Wang YiAnd5.Wang Yi-RaceThe amplificationXbp1-F2,Xbp1-R2AndUPMMixed Primer(Mixed concentration Reference Kit Manual)First-round amplification and then first-round AmplificationPCRProduct

Dilution10Double as the second round, die, plate, and then useXbp1-F3,Xbp1-R3AndNupThe second round of amplification was carried out with primers.20μL PCRReaction System(YaoEt al, 2016). Reaction Procedure:

Pre-denaturation3 min;94Denaturation30 s;68Annealing30 s;

C Extension1 min,35A loop. AmplifiedPCRProduct Sutra1%After agarose gel electrophoresis, it was sent to Beijing Liuhe Hua big Gene company for sequencing. Sequencing results andXbp1-SOfCDNASequence alignment results are shown in Fig.1., Find the start password andPolyAAdd tail signal.

1.2.4 Nile tilapiaXbp1-SSequence analysisWithDNAstar

In softwareSeqmanProgram to clone the carrier, Will3.Wang Yi,5.Wang YiCombination of sequencing resultsCDsSequence splicing analysis, further confirmationXbp1-SAmplification obtainedCDsThe full length. WithNCBIWebsiteBlastSequence Homology analysis of the encoded protein;BioeditSoft

Of Nile tilapia and other fishXbp1-SMultiple alignment of the amino acid sequence; ApplicationMega 6.0Software, with ortho-junction Method(NJLaw)Build a phylogenetic tree andBootstrapRepeat1000Calculate the confidence of each branch. Prediction of protein Physicochemical PropertiesProtparamSoftware(Http://web.expasy.org/protparam), So that

NCBISite conservative domain(CDD)Database(Http:/www. ncbi.nlm.nih.gov/structure/cdd/wrpsb.cgi)Prediction of conserved domains for amino and basic acid sequences.

1.2.5 Nile tilapiaXbp1-SPreparation of polyclonal antibodyAccording

Xbp1-SOfCDsSequence of the zinc finger structure.504 BPProkaryotic Expression Analysis and constructionPET32A-XBP1Protein Expression Vector and importBL21Specific Expression of about40 kDaDestination fragment(ContainsPpet32aMolecular Weight of Carrier). Then, the purified recombinant protein was concentrated with the Freund's adjuvant.Sigma)Mixed, intraperitoneal immune mice(BALB/c, 8Zhou),2.Zhou immunization1.Continuous immunization4.For the first time, Freund's complete adjuvant was used, and the Freund's incomplete adjuvant was used again.50μG/Only. After getting the antiserum, the resistance was detected.ElisaCheck its titer. In the first4.After immunization4 D (Highest antibody titer)The antiserum was obtained from the tail vein for protein detection and immunohistochemical staining.

1.2.6 Nile tilapiaXbp1-SOrganization expressionFluorescence QuantitativePCRMethod detectionXBP1InMRNAExpression Level in healthy tilapia tissues, usingWestern-blotMethod detectionXBP1Protein distribution in different tissues. Take healthy3.The head kidney, posterior kidney, spleen, skin, Gill, intestine, thymus, liver and muscle tissue of tilapia tail Nile-80C. Preservation. And organizationsRNAAnd tissue protein extraction.RT-PCR

Western-blotDetection of Tissue proteins. ForRT-PCRPrimers for detecting the expression in different tissues of Nile tilapia1.. Reaction Conditions:

95C3 min;95C1 min;60C30 s;72C30 s,35A loop. Repeat for each sample3.Time, accordingC_T(Threshold cycles)

Value(15)<C_T<35)ToBeta-ActinGene as internal reference, using formula2.$^{Wang\ YiCT}$Draw each sampleXBP1Relative expression of genes.

1.2.7 Streptococcus agalactius stress testTake60Niro Nakai

Tilapia randomly divided2.Stress group and control group. Intraperitoneal injection in stress group100μLConcentration is5x10^5·Cfu/μLStreptococcus agalactiae(Tellez-BañuelosEt al, 2009; GaNEt al, 2015)Intraperitoneal injection in control group100μLSterilePBS, Injection0,6.,24,48,192,264 h.After treatment, the stress group and the control group were randomly selected3.The tail fish is frozen in liquid nitrogen and preserved in-80Refrigerator for subsequent organizationsXBP1Gene'sRT-PCRAnalysis and detection.RT-PCR

1.2.8 Xbp1Immune wasSelect Health Nile tilapia of head kidney white blood cells for immune was experimental. Head renal white blood cells of Separation UsingPercollDensity gradient separation the according to Nile tilapiaBCells of proportion respectively preparationPercoll 40,50,60And70Reagent superposition afterPercollDensity gradient separation liquid to separation development of differentiation degree differentBCells subclass. In super-clean workbench take health Nile tilapia of head kidney and join3 ml 1640Cell culture medium the full grinding learn supernatant removal big of organization block spare. According to "with manual firstPercoll (GE

Healthcare)Liquid10 PBS (Nah_2PO_4In 2 H_2O 2.286gNa_2HPO_4In 12 H_2O 29.14gNaCl 87.75g) 9:1Dilution for sparePercollStorage liquid then1 PBSFurtherPercoll 40,50,60And70The superposition each layer SeparationBCells. The separation of cells further cells smear afterEnVisionTMImmune organization chemical two-step main steps including: slice with two toluene dewaxing gradient ethanol off benzene to water hydration;3%Hydrogen peroxide solution at room temperature to avoid light Incubation25 minBlocking endogenous peroxidase;3% BSAAt room temperature closed30 minAfter drop Add a,4 ℃ Incubation night; fluorescence two anti-incubated at room temperature30 min; Fluorescence microscope microscopic examination positive cells significantly of fluorescence and the image collection of analysis.

2. Results and Analysis

2.1 OnXbp1-SGeneCDNAFull-length Cloning and Sequence Analysis

According to acquiredORFSequence further design specificRACEPrimersXbp1-F2,Xbp1-F3AndXbp1-R2AndXbp1-R3 (Table1).Xbp1-F2AndXbp1-R2And general primersUPMAndXbp1-F3AndXbp1-R3And general primersNUPRespectively pair

3 -RaceAnd5 -RaceAmplification(YaoEt al2016)Of products were confirmed by sequencing Panel after get Nile tilapia gene full-lengthCDNASequence

1155 BP3 End non-coding region(3 'UTR)Long98 bp5 End non-coding region(5 UTR)Long127 bp3 ContainsPolyATail and more poly adenylateAataaAnd tail Signal(Figure1). Amino acid sequence analysis shows that,OnXbp1-SGene can Coding384A amino acid residues the derivation of molecular weight41.32 kDaTheory isoelectric point4.36. By protein of function forecast indicates that,OnXbp1-SContains174 bpOf zinc refers to structure regional(Figure1Underline part)The regional is compare the conservative protein structure the protein belongs to alkaline leucine zip structure protein.

2.2 OnXbp1-SGene of homology analysis

UseBioeditSoftware of Nile tilapiaXbp1Gene encoding the amino acid sequence and red takifugu, rainbow trout, and the European food with Mu Li(Ostrea edulis), Spot zebrafish, spots bird eel(Lepisosteus

Oculatus), Mexico fat carp(Astyanax mexicanus), Green (Oryzias latipes)(Oryzias latipes), Antarctic cod(Notothenia coriiceps), Jungle Fowl

(Gallus gallus), Mice(Mus musculus)And people(Homo sapiens)The homologous comparison and different species in zinc refers to the structure of homology up79% (Figure2). As shown in figure2Shown in black shadow said homology greater75%Of Regional Main is concentrated in different species of zinc refers to the structure of conservative regional. UseMEGA 6.0Software to have published of lactation animal and common of fish the system evolution analysis display Nile tilapiaXbp1And Antarctic cod belong to a branch homology highest and other invertebratesXbp1Together for a class. But there are obvious differentiation in mammals, amphibians, birds and fishes.(Figure3).

2.3 Expression and Purification of prokaryotic protein and preparation of Its Polyclonal Antibody

Nile tilapiaOnXbp1-SGene Fragment as template, using specific primersXbp1-F4,R4 (Table1)Successfully amplified to length

BPTo connect the destination fragmentPET32aPlasmid, obtain the recombinant plasmidPET32a-Xbp1. Transfer recombinant plasmid into Escherichia coliBL21 (DE3)After induction, the positive clones were sequenced correctly and the expression of the target protein before induction was weak.(Figure4)The size of the bands that were expressed after induction was about40 kDaConsistent with the predicted molecular weight of the fusion protein(Including target protein18 kDaAndPET-32aTag Protein21 kDa). The results of Soluble analysis showed that the fusion protein mainly existed in the form of inclusion bodies. JingSDS-PAGEDetection, purification of proteins in40 kDaThere is a

single band. With ResistanceHis-tagMonoclonal Antibodies AgainstWestern-blotThe purified protein was detected in

KDaThere is a single band atOnXbp1-SExpressed fusion protein. The purified protein was concentrated, and the mice were immunized with antigen and antiserum was obtained.ElisaThe antibody titer was800,000 (units/ml)Showed that the prepared mice were resistant to tilapia

Xbp1-SPolyclonal antiserum had good effect.

2.4 Nile tilapiaXbp1-STissue Distribution of gene and protein levels and stress expression of Streptococcus

AdoptedReal-time qpcrMethod detectionOnXbp1-SMRNAExpression in different tissues of healthy Nile tilapia;

PreparedOnXbp1-SPimaWestern-blotDetectionOnxbp1

Protein Expression levels in different tissues.MRNAThe results of relative expression showed that,OnXbp1-SIn the Detected8.The expression in tissues, from low to high, followed by muscle<Thymus<Spleen<Skin<Gill<Chang<Head kidney<The highest expression was found in the liver and in the head kidney.

After intraperitoneal injection of streptococcus, compared with the control group, the liver and spleen were up-regulated at different time points, and the trend was basically the same. Early intraperitoneal injection6 hAfter,OnXbp1-SGene expression was significantly increased, and24 h

48 hThe expression192 h.To the highest level of Gene Expression(Figure6a, Figure6b)And then in264 h.Gradually dropped down and tended to the level of the control group.

2.5 Nile tilapiaXbp1-SAt differentBImmunohistochemical analysis of cell subtypes

AccordingZwolloWait.(2005)Reported.PercollTilapia head kidney was isolated by density separation method.BCells:Percoll 40Cell(Plasma cells, fig.7A),Percoll 50Cell(More than puree

Cell, graph7B),Percoll 60Cell(Initial differentiation matureBCell, graph7c), AndPercoll 70Cell(Mostly immatureBCell, graph7D). As shown in Fig.7.As shown,OnXbp1-SProtein in differentBThe expression level of the cell subclass can be shown by its red fluorescence.

Change from low to high isPercoll 70 <Percoll 60 <Percoll 50 <Percoll 40OnXbp1-SThe highest expression in plasma cells, the highest level of expression in primary plasma cellsBCells have a little expression, but in immatureBCells are barely expressed.

3. Discussion

Cell Transcription FactorXBP1Expression andBThe development and differentiation of cells and the expression and secretion of antibodies are closely related.(ZwolloEt al, 2005,2008; TELLIEREt al, 2016).XBP1It is one of the major mediators of protein folding ability in endoplasmic reticulum stress.2.Different types of cuts(Xbp1-SAndXbp1-U)Where,Xbp1-SHighly transcribed(ZhouEt al, 2008). Endoplasmic Reticulum regulates protein folding, post-transcriptional modification gene expression and other functions,If a wrong folding occurs, it will lead to an anti-apoptotic reaction and promote the unlimited proliferation of tumor cells.(Hendershot, 2004; RobinsonEt al, 1994). Therefore,XBP1ResearchBThe development, differentiation and regulation of Cells and Their Immune Function in the defense of pathogenic bacteria infection are of great significance.

This study cloned Nile tilapiaOnXbp1-SAnd the prokaryotic expression system of exogenous protein was used.(SalinasEt al, 2011)Successful buildOnXbp1-SIn Vitro Expression System, polyclonal antibody was prepared,OnXbp1-SIn normal tissues of tilapia niloticus; in the stress period of Streptococcus,OnXbp1-SThe expression in liver and spleen was up-regulated and the reaction tended to be synchronous.OnXbp1-SFor plasma cells that are matureBRegulation of cell development and differentiation.

XBP1It is widely expressed in mammalian tissues, especially in hepatocytes.(LiouEt al, 1990; calfonEt al, 2002). The results showed that Nile tilapiaOnXbp1-SIt is expressed in various detection organizations.MRNA

The level is mainly expressed in the liver, which is similar to the discovery in mammals. However, high protein

expression on the protein level is mainly in the thymus and head kidney, suggesting thatXbp1-SEXPRESSION AND IMMUNE CELLS(B)Cell andTCell)There may be some correlation(ZwolloEt al, 2005,2008; TELLIEREt al, 2016). Development and differentiation of the Main Immune Cells in the immune systemXbp1-SThe positive correlation of expression reflectsXbp1-STranscription factors may play an important role in the immune response, becauseXbp1-SCytokines are not only involved in humoral immunity, but also may be involved in the promotion of cellular immunity. Streptococcus agalactius stress test showed that after stress264 h.During,OnXbp1-SIt was up-regulated in spleen and liver. Intraperitoneal injection of Streptococcus6 hAfter the liver and spleen4) OnXbp1-S mRNAThe first peak was a significant increase in expression, which indicated that this factor might be involved in the natural immune response. The second more significant expression peak occurred after Streptococcus stress.192 h (8 d), This significant increase is likely due to immune cells involved in the adaptive immune response(B)Cell andTCell)ChinaXbp1-SThe expression of factor was significantly up-regulated.

The head kidney of the bony fish is similar to the bone marrow of mammals, having hematopoietic function and is a site for the development of immune cells. Therefore, the head kidney tissue contains immature blood cells of different blood lines.(FengeEt al, 1986; ZapataEt al, 1995). The head kidney is an important place for the occurrence, development, differentiation and proliferation of immune cells.

Organ(KaattariEt al, 1985; bromageEt al, 2004; yeEt al, 2010; MAEt al, 2013). In this study, the head kidney tissue of Nile tilapia wasPercollDensity gradient separation method to obtain different developmental DifferentiationBCell subclass(ZwolloEt al, 2005)Immunohistochemical staining was used to detect the fluorescence intensity of cells.OnXbp1-SHighly expressed in mature plasma cells and protoplasmic cells with high degree of differentiation.Naïve BCells and immatureBThe expression of cells was low. Nile tilapia

Cell subclasses expressed at different levelsOnXbp1-SPhenomenon, with the reported red fin takifugu(OhtaniEt al, 2006)And rainbow trout(ZwolloEt al, 2008)Similar.BCell Differentiation andXbp1-SThe positive correlation of factor expression indicates that this factor plays an important role in promoting the development of mature plasma cells andBCell Differentiation plays a positive role in regulating the differentiation of protoplasmic cells.(YeEt al, 2011).Xbp1-SImmune Function especiallyBThe role of cell differentiation is conducive to the exploration of humoral immunityBRegulation Mechanism of cell development and differentiation. In conclusion, this study will provide a basis for further study of Nile tilapia.Xbp1-SMechanism and promotion of factor response to pathogen infectionBProvide a theoretical basis for cell differentiation mechanism.

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