Cloning and the Expression of Insulin Growth Factor-II and the Analysis of its Function Research

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Abstract: The insulin-like growth factors (IGFs) I and II are polypeptide hormones related to insulin by structure and function which contribute to the mitogenic properties. In vitro, the mitogenic activities of IGF-I and -II are similar, but in vivo they are different. IGFs are multiple proliferation controlling factors of cells. It is necessary for normal fetal growth and development. In fact, the main function of IGF-II in human is not clear. In our research we got the cloning active IGF-II, and analyzed and predicted the structure and function of IGF-II with bioinformatics. [Journal of American Science 2009;5(5):13-16]. (ISSN: 1545-1003).

Key words: Insulin-like growth factor-II (IGF-II); His-tag; bioinformatics; domain

1. Introduction

Insulin-like growth factor-II (IGF-II) is a protein with 67 amino acids. Its molecular weigh is 7.4kD. Human IGF-II locates on chromosome 11 and close to insulin. It has nine extrons and four promoters. IGF-II also has four domains, they are domain B,C,A and D. IGF-II plays essential roles in cell metabolism, proliferation and differentiation and to this extent have major effects on fetal and postnatal development and organogenesis in mammals(Underwood, 1984; Humbel, 1990).The main function of IGF-II in humans is not clear. In rodents, IGF-II may be the function as a fetal growth factor; the levels of IGF-II in fetal rat plasma are high and decline after birth.

IGF-II values in adults are about four times higher than those of IGF-I(Vorwerk, 2002). Recently, the studies of IGF-II are associated with its imprinted gene. Genomic imprinting is a gene regulation method whereby a gene is expressed in a parent-of-origin dependent fashion(Catherine, 2001). Paternal expressed IGF-II encodes a critical protein for fetal mitogen, and mice deficient of this growth factor have a dwarf phenotype. IGF-II and H19 are closely linked(Hemberger, 1998; Bartolomei, 1991; Barlow, 1991) imprinted genes located at the centromere end of 1Mb imprinted domain on mouse chromosome 7(Ishihara, 2002). They are expressed only from the paternal and the maternal allele, respectively.

2. Experimental procedures

2.1 Materials and Reagents

Human placenta: Harbin Red Cross Center Hospital. JM109: Takara biotechnology (Dalian) co, ltd. Plasmid: pGEM-T-Easy; Amp resistance; pET30a (+); Kna resistance, Promega co, ltd.

2.2 Cloning, Expression and Purification of target protein

The RNA was isolated by Trizol extraction from human placenta(Haselbacher, 1987). According to the recorded insulin-like growth factor-II gene in GenBank (NM000612), we designed the primers with the restriction enzyme sites of *NcoI* and *XhoI*, RT-PCR amplification of target gene. Use T_4-DNA Ligase to link the PCR product and pGEM$^+$-T-Easy Vector. Transformed the linkage products into E.*coli*JM109 competent cell. Then pave on the flat plate together with X-gal, IPTG for resistance test. Pick out recombinants and inoculate into LB medium with 50mg/ml Amp, 37□ culture about 12 hours, isolated plasmid DNA, restriction enzyme verification for the recombinants plasmid and sequence analysis. The result of the sequencing is the right gene we cloned.

Then construct fusion expression vector, cutted the IGF-II-pGEM$^+$-T-Easy and the pET30a (+) use *NcoI* and *XhoI*. Link IGF-II fragment and

pET30a(+). Transform the linkage products pET30a (+)-IGF-II into E.*coli*JM109 competent cell; screen the recombination plasmid with Kna resistance. Isolate the plasmid, by restriction enzyme and PCR verification. The expression of IGF-II fusion protein, we induced the transformed JM109, inoculate it into the LB medium with Kna, 37℃, stay over night, and then inoculate the bacterial in the fresh LB medium with Kna, 37℃ cultivate till the OD is 0.6. Add IPTG till the final concentration is 1.0 mM, induced 2 hours and 4 hours respectively, 12000rpm centrifugation one minute, discard the supernatant, add 100μl sterile water to suspend the sediment and then add the buffer boiling for three minutes. SDS-PAGE According to the *molecular cloning* protocol.

Due to the target is in the sediment, it needs to wash the inclusion body, then to do the His-tag Affinity Chromatography.

2.3 Insulin-Like Growth Factors Bioinformatics Research

2.3.1 Structure of the Insulin-Like Growth Factor-II Prediction

Utilized the bioinformatics software and Chou-Fasman method to predict and analyze the secondary structure of IGF-II. Chou-Fasman method is an experiencing parameter method based on the statistic of single amino acid residue. Through the statistic analysis, it can be obtained the proneness to present the specific secondary structure of each single amino acid residue, which can be used to predict the protein secondary structure(Sunxiao, 2005).

2.3.2 Function of the Insulin-Like Growth Factor-II Prediction

According to the homology comparison model, analyze the domain of the insulin-like growth factors family; search the active and binding sites of the receptors and the binding proteins. Further, analyze and predict the correlative functions of IGF-II.

3. Result

3.1 Abstraction of human placenta total RNA

The total RNA from fresh human placenta was isolated by TRIzol Kit (QIAGEN). Dissolve the RNA sediment with DEPC treated water. OD_{260}/OD_{280}=1.8, shows the good purity of RNA (Fig.1A).

3.2 PCR amplification of IGF-II

We amplified a specific DNA band with the length of 218bp, which is conformity with the expectation, under the grading-up PCR system (Fig.1B).

3.3 Isolation of recombination plasmid and PCR verification

Link the PCR products which have *NcoI* and *XhoI* cohesive end to the pGEM$^+$-T-Easy Vector (Fig.1C). Then transform the linkage products into *E.Coli*JM109. Cultivate them on the LB medium which have Amp+ X-gal+ IPTG over night. Pick out the recombinants and inoculate into LB medium with 50mg/ml Amp cultured, isolate plasmid DNA, restriction enzyme verification for the recombinants plasmid (Fig.1E) and PCR verification. We obtained the target gene with the length of 218bp (Fig.1D). It is shows that the target gene was already inserted into the plasmid.

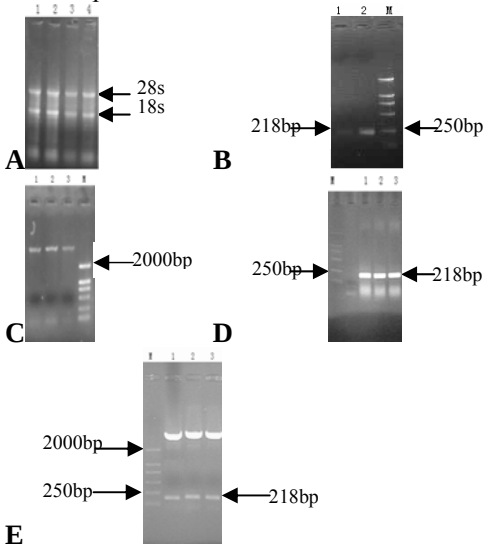

Figure.1 A. Abstraction of total RNA from placenta. B. PCR Agarose gel electrophoresis analysis of IGF-II gene. 1: 50℃, 2: 53.3℃, M: DL2000marker. C. Agarose gel electrophoresis analysis of linking product. 1-3: linking products, M: DL2000marker. D. Identification of recombinant Plasmid pGEM$^+$-T- IGF-II by PCR. 1-3: PCR plasmid, M: DL2000marker. E. Identification of recombinant plasmid pGEM$^+$-T- IGF-II by restriction enzyme *Nco*I, *Xho*I. 1-3: target gene, M: DL2000marker.

3.4 Sequencing and alignment

We obtained the target gene (accession number is EU622024) with 99.5% of known sequence homology (Fig.2).

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     GCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGAGCTGG    40
NM   GCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGAGCTGG    40
     gcttaccgccccagtgagaccctgtgcggcggggagctgg
     TGGACACCCTCCAGTTCGTCTGTGGGGACCGCGGCTTCTA    80
NM   TGGACACCCTCCAGTTCGTCTGTGGGGACCGCGGCTTCTA    80
     tggacaccctccagttcgtctgtggggaccgcggcttcta
     CTTCAGCAGGCCCGCAAGCCGTGTGAGCCGTCGCAGCCCT   120
NM   CTTCAGCAGGCCCGCAAGCCGTGTGAGCCGTCGCAGCCCT   120
     cttcagcaggcccgcaagccgtgtgagccgtcgcagcc t
     GGCATCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGG   160
NM   GGCATCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGG   160
     ggcatcgttgaggagtgctgtttccgcagctgtgacctgg
     CCCTCCTGGAGACGTACTGTGCTACCCCCGCCAAGTCCGA   200
NM   CCCTCCTGGAGACGTACTGTGCTACCCCCGCCAAGTCCGA   200
     ccctcctggagacgtactgtgctacccccgccaagtccga
     C                                          201
NM   G000612                                    201
     g
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Figure.2 The sequencing result of IGF-II and alignment. Up-line: sequencing result, middle-line: known sequence, down-line: consensus sequence.

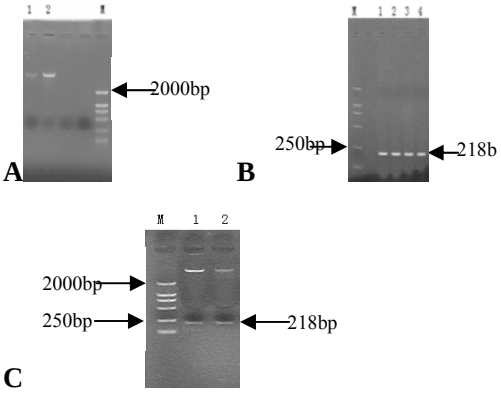
3.5 The fusion expression vector construction and the target protein expression

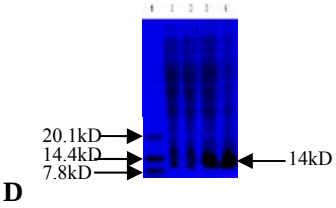
Cut the pGEM^{+}-T-IGF-II and pET30a (+) with restriction enzyme *NcoI* and *XhoI*. Link the cut genes to pET30a (+)-IGF-II and then transform into *E.Coli*JM109 competent cell. Isolate plasmid DNA; make the PCR and restriction enzyme verification (Fig.3A.B.C).

Induce the pET30a (+)-IGF-II with IPTG for 2 hours and 4 hours respectively. Through the SDS-PAGE, we obtained 14kD target protein, including part of fusion protein, the same as we anticipated (Fig.3D).

3.6 The target protein purification

pET30a (+) has 6 His-tag. So we do the his-tag Affinity Chromatography test (Fig.3E).





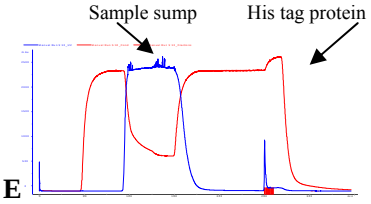


Figure.3 A. Purification of plasmid after transformation. 1-2: PET-30a (+) - IGF-II. B. Identification of recombinant plasmid PET-30a (+)-IGF-II by PCR. 1-4: PCR result, M: DL2000marker. C. Identification of recombinant plasmid PET-30a (+)-IGF-II by restriction enzyme *Nco*I, *Xho*I. 1-2: PET-30a (+)- IGF-II, M: DL2000marker. D. SDS-PAGE of PET-30a (+) -IGF-II. 1-2: not induced, 3: IPTG induced for 2h, 4: IPTG induced for 4h, M: SDS-PAGE protein marker. E. Purification of the target protein.

3.7 The research on insulin-like growth factors with bioinformatics

3.7.1 The prediction of the structure of IGF-II

Through the bioinformatics analyses, we obtain the following result: 1-15, 21-35, 38-42, 48-54, 58-67 amino acids are prefer to form the structure of Coil; 16-20, 43-47, 55-57 amino acids are prefer to form the structure of Strand; 36-37 are prefer to form the structure of Helix.

3.7.2 The prediction of the function of IGF-II

According to the homology comparison model, analyze the domain of the insulin-like growth factors family, to search the reaction sites of the factors with receptors and the binding protein. Further, to analyze and predict the correlative function of IGF-II. IGFs have 4 chains: B, C, A and D. IGF-I、II have very high homology in A and B chain. According to the homology comparison, No. 23, 24, 25, 60 amino acids of IGF-II are same as the ones of IGF-I. These amino acids are the reaction sites of IGF-I with IGF-IR. Therefore we can predict that IGF-II has the same reaction site with IGF-IR and have the similar function.

No. 3, 4, 5, 9, 12, 16, 52, 53, 54, 57, 58 amino acids of IGF-II are same as the ones of IGF-I. These amino acids are the reaction sites of IGF-I with

IGFBP-5. Therefore we can predict that IGF-II has the same reaction site with IGFBP-5 and have the similar function.

Some researches showed that IGF-I can mediate hormonal dependent inhibin expression and steroidogenesis through the development of dominant ovarian follicle. Granule cells can create IGFBP-5, which can inhibit this effect and lead to the atrophy of un-dominant ovarian follicle. Therefore we can predict that IGF-II interact with IGFBP-5 to have the similar function as IGF-I.

Also IGF-I interacts with IGF-IR to promote the growth of chondrocyte. So maybe IGF-II can interact with IGF-IR and has the similar function as IGF-I.

4. Discussion

Insulin-like growth factors are the very important growth factors. They interact with the receptors and the binding proteins. Insulin-like growth factors play very important regulated roles in the cell proliferation, growth and pathogenesis of cancers.

There are not so many researches on IGF-II, therefore the study on IGF-II can supply a helpful function during not only the science research but also the clinical research.

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