GW23-e0852 **CONSTRUCTION AND IDENTIFY OF PROKARYOTIC VECTOR OF TIMP-3**

doi:10.1136/heartjnl-2012-302920a.286

Duanlianxin Sunxin, Tiandan Gaoxin, A Sunxin. *Beihuda University*

Objectives constructing prokaryotic vector of Timp-3 (tissue inhibitor of metalloproteinase-3) and identify it.

Methods The total RNA was extracted from human placenta tissues, amplify the gene by RT-PCR and the fragmental gene connect T vector, then clong. And cut Timp-3-pMD18-T by BamHI and XbaI. Agrose gel electrophoresis observe the result. After cut by BamHI and XbaI, enzyme must succeed, sequencing the same like Timp-3 in CNKI, This plasmid was reserved into *E. coli* DH5α.

Results By agrose gel electrophoresis identification: We can get Timp-3 gene fragment after cut Timp-3-pMD18-T. By Sangon Biotech (shanghai) Co. Ltd. Sequence, the result same to Gene Bank's Timp-3 gene, and approve right for construction and identify of prokaryotic vector of Timp-3.

Methods The total RNA was extracted from human placenta tissues. The first, we have prepare RNA-free water, and use RNA-free glass bottle, put DEPC in ultra pure water to 0.1% final concentration, after agitate pass the night, autoclave sterilisation. Secondly, We have collect 0.1 g tissue that the tissue is pulv human placenta, after mix 500 ml of RNAAiso plus, homogenate, quiescence at room temperature for 5 min, add to chloroform of 1/5 RNAAiso Plus volume, joggle and durchmischung for 15s. After quiescence at room temperature for 5 min, 12 000 r/min centrifugal 15 min at 4°C. Make supernatant into the new append off tube. Add equal volume isopropanol the same as supernatant, quiescence at room temperature for 10 min, 12 000 r/min centrifugal 10 min at 4°C. Abandon supernatant, put 75% alcohol into sedimentation with 1 ml to purge sedimentation, 12000 r/min centrifugal 15 min at 4°C. Abandon supernatant and keep the sedimentation, and evaporate sedimentation for 5–10 min in the air, then add disposal

water by DEPC with 20 μl, we have get total RNA. Then by agrose gel electrophoresis identification, and keep total RNA to −80°C. in reserve primer express

Device sense and antisense restriction enzyme cutting site, they are BamHI, XbaI. Sense: 5′-CGGGATCCATCCCCTTGGCT CGGGCTCATC-3′; antisense: 5′-GCTCTAGAGGGGTCTGTGGC ATTGATGATGC-3′. This primer synthetise by Sangon Ltd. of Shanghai.

RT-PCR

$Mgcl_2$ 2 μl, 10×RT Buffer 1 μl, dNTP 1 μl, RNase Inhibitor 0.25 μl, AMV 0.5 μl, oligodt 0.5 μl, total RNA 4 μl, we use disposal water by DEPC to 10 μl, durchmischung. 42°C 30 min, 99°C 5 min, to compose the first chain of cDNA. At the moulding board of reverse transcription outcome, add 5×PCR Buffer 10 μl, Taq enzyme 0.25 μl, sense 0.5 μl, untisence 0.5 μl, we use disposal water by DEPC to 50 μl amplification PCR: rise in temperature for 2 min at 94°C, 94°C 30 s, 55°C, 30 s, 72°C 90 s, amplification 30 circles, stretch for 7 min at 72°C. Then by agrose gel electrophoresis identification to PCR production, and observed result.

Cut by BamHIand XbaI

Get above DNA 1 μl, BamHI1 μl andIfor 1 μl, 10×KBuffer 1 μl, high pressure double distilled water 13 μl, put it for 3 h at 37°C. Then, agarose gel DNA fragment purification by agrose gel electrophoresis identification, and observed result.

TA linkage and conversion

pMD^{TM}18-T Vector 1 0.5 μl, InserDNA3 4.5 μl, solutionI5 μl, total volume is 10 μl. Put above liquid in competent cell of 200 μl, and put all liquid on ice for 30 min. Mix LB culture media of 1790 μl, oscillating culture for 1 h at 37°C, 4000 rpm centrifugal 10 s, abandon supernatant, by LB culture media of 200 μl suspend again. Put bacterial liquid shop in LB agar plate contain ampicillin, spread on everywhere, put it 20–30 min at room temperature, and culture it for 12 h to 16 h in couveuse at 37°C.

Screening bacterial colony

Picking monoclone bacterial colony after conversion, and make the germ a lot of proliferation, then we have extract plasmid. The plasmid is cut by BamHIand XbaI, agrose gel electrophoresis identify product after cut by BamHIand XbaI, sequencing by Sangon Ltd. of Shanghai, continue to have the culture.

Results Picking total RNA

Picking total RNA with trizo reagents, the result by agrose gel electrophoresis is figure 1.

Appraisal of amplificative production by RT-PCR.

Total RNA of human placenta tissues amplificative through RT-PCR, we can see specificity band by agrose gel electrophoresis in the shadow of 633 bp, equal to expect segment magnitude as figure 2.

After link Timp-3 gene and pMD™18-T, cut this, as Fig.3. Through BamHIand XbaI were cut, by agrose gel electrophoresis, we can see clear band in the shadow of 2692 bp and 633 bp as figure 3.

By Sangon Ltd. of shanghai sequencing, as in figure 4.

Appraisal of amplificative production by RT-PCR

Total RNA of human placenta tissues amplificative through RT-PCR, we can see specificity band by agrose gel electrophoresis in the shadow of 633 bp, equal to expect segment magnitude as figure 2.

After link Timp-3 gene and pMD™18-T, cut this, as figure 3. Through BamHIand XbaI were cut, by agrose gel electrophoresis, we can see clear band in the shadow of 2692 bp and 633 bp as figure 3.

By Sangon Ltd. of shanghai sequencing, as in figure 4. through inhibit MMPs' activity and induced cells apoptosis' functional locus isolated N-extremity structural domain, C-extremity structural domain have function to combine ECM.[6,7] Because of these function, constructing prokaryotic vector of Timp-3 this experiment, Our studies aim to research function of atheroma, treat tumours to lay a foundation, through inhibit MMPs' activity and induced cells apoptosis' functional locus islocated N-extremity structural domain, C-extremity structural domain have function to combine ECM.[6,7]

Conclusions Timp-3 is natural inhibit MMPs, Timp-3 can through inhibit MMPs' activity,[8] to stifle the tumours invasion, proliferation, blood vessel of development and metastasis. In addition to stable TNF-αreceptor, Fas, and promote the fas induced cells apoptosis.[4,5] Timp-3 can through inhibit MMPs' activity and induced cells apoptosis' functional locus isolated N-extremity structural domain, C-extremity structural domain have function to combine ECM.[6,7] Because of these function, constructing prokaryotic vector of Timp-3 this experiment, Our studies aim to research function of atheroma, treat tumors to lay a foundation.