Expression of Human Intestinal Trefoil Factor in *Pichia pastoris* and Its Biological Activity on Intestinal Epithelium in vitro

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Abstract In order to produce relatively large amounts of recombinant human intestinal trefoil factor and assess its biological activity. The expression plasmid pPIC9-hITF containing AOX1 promoter and the sequences of secreting signal peptides was transformed into the yeast cells. Then through selection positive transformants were cultivated in fermentation basal salts medium in a 5L fermenter to obtain large amount product with low cost. The secreted peptides were then purified by a combination of ionic exchange chromatography and molecular sieve. To verify the product electrospray mass spectrometry analyses was used to determine the structure of rhITF and Western Blotting was performed to test the immunological activity. Furthermore the biological activity of the peptide was examined by experiments from cell to tissue. The nucleotide sequence of rhITF was the same as expected. With a 5-L fermenter 253 mg of hITF was isolated at the purity of 96% from 3.5L of yeast fermentation broth. The expression level for recombinant human ITF in this yeast system was 73.33 mg/L. In our study we provided a way to gain a production among milligram to gram of recombinant human ITF by the use of a yeast expression system. As human ITF are difficult to purify in any significant amount from tissue extraction the way described may become a valuable tool in obtaining pure peptide for further studies of trefoil peptide function.

Key words human intestinal trefoil factor *Pichia pastoris* expression cell migration
我们利用基因工程技术,从人结肠粘膜中克隆
量极低,直接从组织中分离
基因,构建

培养基:
动物有限公司提供,动物批号证书:沪动
自北京协和医科大学细胞保藏中心。

限制性内切酶、
反应试剂和

\[ 4 \text{S R} \]

化仪器(

化,乙醇沉淀回收线性化质粒,采用电击法转化到

凝胶电泳后,

体及目的基因片段均用

反应条件为

和

酶切,酶切产物分别经

对大鼠肠上皮细胞迁移的影响:

取

沉淀,精洗洗脱峰,并采用

法

的性质,实验主要由上海第二医科大学病理生理教

验

的缓冲液进行梯度洗脱,

采用大鼠小肠上皮细胞株(

公司的

和宿主菌中,电击产物涂布于

的重组质粒

的重组质粒

及

重组质粒的构建、转化及转化子筛选和

测定

递表达目的蛋白质。以

酶

和

目的基因片段均用

酶

用胶回收试剂盒回收目的基因片段并用

的重组质粒

的重组质粒

重组质粒经上海基康

的真核表达载体,将载

生物学活性鉴定:

测定

鉴定:质粒


cDNA

合成

Xho

目的片段筛选阳性转化子。从

取

延伸

得

在

对大鼠小肠上皮细胞

电

验证


1

1.1

1.1.1 pPIC9

GS115

Mut‘

his4

Invitrogen

TG1

LaCento

PCR

GIBCO BRL

Bio-Rad Gene Pulser

Bio-Rad

BioFlo III

NBS

Sepharose G-25

Pharmacia

CHEMICON

IEC-6

SD

5 - 6

180 - 220

BK

152

Sigma

MPO

1.1.2 MD

13.4

100mmol/L

pH6.0

YMBY

3

20g/L

YMBY

4

13.4

3.5

4ms/cm

Sepharose G-25

MonoQ

S-100

BMY

1000mL

NaCl

Lowry

MALDI-TOF-TOF-MS

1.2

1.2.1 RNA

hITF

cDNA

Ph

5’TGCAGCTCGAGAAAA

GAGAGA...

Not

P2

5’TGGCCGCCGACTGCTGGCAGAACATGACAG-3’T

PCR

94°C 3min

94°C 30s

57°C 45s

72°C 30s

72°C 5min

PCR

2%

PCR

1%

Sal

GS115

MD

30°C

2 ～ 3d

Invitrogen

PCR

20

BMY

1.2.2 hITF

DNA

pPIC9

Xho

Not

pPIC9-hITF

Sal

1.2.3 hITF

BMY

1.2.4 hITF

1000mL

4ms/cm

SP-Sepharose G-25

MonoQ-Sepharose S-100

1mol/L

NaCl

0 ~

100%

Lowry

MALDI-TOF-TOF-MS

1.2.5 hITF

IEC-6

Chemicon
司细胞迁移试剂盒使用说明书进行。以无血清培养基培养细胞，造成细胞饥饿后，以胰酶消化制备细胞悬液，细胞浓度为1×10⁶/mL。PBS 10⁻⁵ mol/L hITF 14h。每个内置孔板(底部为孔径0.2cm×0.5cm的膜，上皮细胞可从孔中穿过)中加入细胞悬液，同时加入0.12或0.01%的重组#678，内置孔板外加入含胎牛血清的培养基，培养后，吸出内置孔内的细胞悬液，将膜底部外侧的细胞洗脱下来，进行荧光染色后，采用荧光酶标仪，波长650nm检测。实验组加入0.01%的重组蛋白，对照组为0.12。

1 RT-PCR

<table>
<thead>
<tr>
<th>腺体</th>
<th>黏液</th>
<th>肉样</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>2.5</td>
<td>1.25</td>
</tr>
<tr>
<td>0.01</td>
<td>1.5</td>
<td>0.75</td>
</tr>
</tbody>
</table>

1.2.6 Student t检验 P < 0.05

2

2.1 RT-PCR

![](image)

Fig. 1 PCR production of rhITF

1 marker 100bp DNA ladder 2 hITF.
2.2 Agarose electrophoresis of expression vector pPIC9/hITF with XhoI /NotI enzyme cleavage

2.3 Different time after the induction of methonal

2.4 Different time after the induction of methonal

2.5 Tricine electrophoresis showing purified protein
1 protein before purified 2 protein after purified 3 hITF standard 4 marker.

2.6 The molecular weight of recombinant protein was determined by electrospray mass spectrometry analyses
35% ~ 45% SDS-PAGE 5 45 Lowry 550 µg/mL 6 73.33 mg/L

2.4

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chain 2 solution structure of the disulphide-linked dimer of human intestinal trefoil factor</td>
<td>gi</td>
</tr>
<tr>
<td>2</td>
<td>Chain A high resolution solution structure of human intestinal trefoil factor</td>
<td>gi</td>
</tr>
<tr>
<td>3</td>
<td>Trefoil factor human Intestine peptide partial 51 aa</td>
<td>gi</td>
</tr>
<tr>
<td>4</td>
<td>TFF3 protein Homo sapiens</td>
<td>gi</td>
</tr>
</tbody>
</table>

Table 3 The result of recombinant protein was determined by electrospray mass spectrometry analyses

<table>
<thead>
<tr>
<th>Rank</th>
<th>Protein name</th>
<th>Accession No.</th>
<th>Protein Score</th>
<th>Protein C. I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chain 2 solution structure of the disulphide-linked dimer of human intestinal trefoil factor</td>
<td>gi</td>
<td>47168540</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Chain A high resolution solution structure of human intestinal trefoil factor</td>
<td>gi</td>
<td>12084578</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Trefoil factor human Intestine peptide partial 51 aa</td>
<td>gi</td>
<td>385570</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>TFF3 protein Homo sapiens</td>
<td>gi</td>
<td>17389674</td>
<td></td>
</tr>
</tbody>
</table>

![Image](image1)

![Image](image2)

![Image](image3)

Fig. 7 The specimen A and microscopic pathology B of colon in rats treated with NS

![Image](image4)

![Image](image5)

Fig. 8 The specimen A and microscopic pathology B of colon in rats treated with 1mg/kg rhITF

2.5 rhITF

2.5.1 4 ! P < 0.05

Table 4 The role of rhITF in the migration of intestinal epithelial cell

<table>
<thead>
<tr>
<th>Control</th>
<th>ITF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average ± SD</td>
<td>41.08 ± 15.14</td>
</tr>
</tbody>
</table>

| P | 0.0478 |

2.5.2

1 | 2 | 4 | P < 0.05 |

Table 5 Effect of rhITF on macroscopic score in rats pretreated with rhITF at 30 minutes before administration of TNBS

Comparison between control group and 1mg/kg rhITF one

| U | 98 |

| P | 4.33E-05 |

2 | 4 | 8 |


本全层粘膜坏死,腺体完全破坏,大中性粒细胞和淋巴细胞浸润,粘膜下层出血,肌层组织发生蜂窝织炎和组织坏死,少数标本粘膜层充血水肿,出血,部分坏死,腺体结构部分破坏,粘膜下层出血,大量炎细胞浸润;预先灌服的实验组标本仅浅层粘膜坏死,脱落,表浅糜烂形成,残留粘膜底层及腺管,腺体结构尚存,局部腺上皮增生,粘膜下层充血水肿,中性粒细胞、淋巴细胞等炎性细胞浸润,粘膜组织损伤程度明显比对照组轻。而且预先给予的实验组病理评分结果同对照组相比均有显著统计学差异(表6)。

表6  Effect of various concentrations of rhITF on microscopic score in rats pretreated with rhITF at 30 minutes before administration of TNBS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1mg/kg rhITF group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO content</td>
<td>5.22 ± 3.40</td>
<td>2.32 ± 1.39</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.030911</td>
</tr>
</tbody>
</table>

3

intestinal trefoil factor

rhITF能够促进肠上皮细胞的迁移。动物实验结果显示,同对照组相比,给予组动物的肠粘膜损伤明显减轻,大体标本和病理组织学评分以及髓过氧化物酶活性均明显低于对照组。髓过氧化物酶是中性粒细胞嗜天青颗粒释放的过氧化物酶类,是保护肠粘膜的作用。
rhITF

1mg/kg

cDNA pPIC9/hITF

hITF

pPIC9/hITF

REFERENCES


