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Immunopharmacology 32 (1996) 96–98

Immunopharmacology

Molecular cloning and tissue distribution of mouse protein C inhibitor (PCI)

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Keywords: Protein C inhibitor; Genomic and cDNA sequence; Tissue distribution

1. Introduction

Human PCI is a non-specific heparin dependent serine protease inhibitor (SERPIN), originally described in plasma as an inhibitor of the anticoagulant serine protease activated protein C and thought to be involved in the regulation of coagulation (Marlar and Griffin, 1980).

Serine proteases inhibited by PCI include thrombin (Suzuki et al., 1984), factor Xa (Suzuki et al., 1984), factor XIa (Meijers et al., 1988), plasma kallikrein (Meijers et al., 1988), urokinase (Geiger et al., 1989), tissue kallikrein (Ecke et al., 1992) and acrosin (Zheng et al., 1994). However, its precise biological function has still not been defined.

A human cDNA for PCI has been isolated and characterised and it has been shown that PCI belongs to the superfamily of serpins (Suzuki et al., 1987). The human genomic DNA of PCI has also been analysed and it has been shown that the gene is 11.5

kbp in length and consists of five exons and four introns (Meijers and Chung, 1991).

Since mouse PCI has not been cloned yet, it was the aim of our study to find out whether a separate PCI entity also exists in mouse.

2. Methods

2.1. Isolation of genomic clones of mouse PCI

A mouse liver BALB/C genomic library in λ EMBL3 (Clontech) was screened with human PCI cDNA obtained by RT PCR amplification from HepG2 cells.

Two independent phage clones (λ EMBL 135 and λ EMBL 150) were obtained and subcloned into pUC 18 and sequenced. These two clones with about 9500 overlapping bp did not contain the complete PCI gene but stop in intron 4.

2.2. Cloning of mouse PCI cDNA

In order to define exactly exon–intron boundaries in the mouse PCI gene, and to PCR amplify a mouse

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gene fragment not present in the genomic clones, cDNA fragments were amplified from a prepared mouse testis cDNA by a modified RACE method and from a purchased λ gt11 mouse testis cDNA library using gene specific primer and a primer in the λ gt11 sequence.

2.3. PCR amplification of a genomic DNA fragment covering the sequence of intron 4 and exon V

In order to gain the mouse PCI sequence not present in λ EMBL 135 and λ EMBL 150 clones and to find an intron 4-exon V boundary, a genomic DNA fragment spanning from exon III to exon V was PCR amplified by using the information of the cDNA sequence.

2.4. Tissue distribution of mouse and human PCI mRNA

Tissue specific expression of mouse and human PCI was compared by Northern blot analysis using total RNA and 32 P labelled cDNA for human PCI, mouse PCI and rat GAPDH as a positive control.

3. Results and discussion

The organisation of the mouse genomic clone and the deduced amino acid sequence of PCI and its comparison with human counterparts (Meijers and Chung, 1991; Suzuki et al., 1989) is given in Fig. 1. Both genes are composed of five exons (I–V) and four introns (1–4), and the exon–intron boundaries follow the GT-AG rule. The length of the exons in both mouse and human PCI are about the same, whereas the size of the introns is different (e.g. intron I human PCI: 5600 bp, mouse PCI 416 bp). The length of PCI gene from the transcription start site to the polyadenylation site in mouse and human is different (~ 5.0 kb versus 11.4 kb, respectively).

The results of tissue distribution of mouse and human PCI are given in Fig. 2. PCI mRNA was detected in many human organs including liver, heart, pancreas, ovary and testis, with the highest concen-

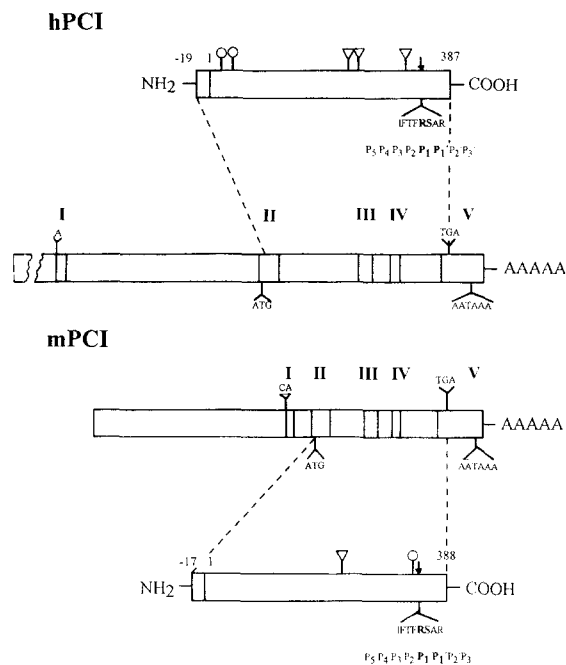


Fig. 1. Gene organisation. Mouse PCI compared to human PCI. The mouse PCI gene encodes a polypeptide of 405 amino acids (comparable to 406 amino acids in human). Like in human, the first mouse exon is noncoding. The putative reactive centre consisted of amino acids RS is located in exon V within the same amino acid sequence IFTFRSAR (named as P5 to P3') in both mouse and human, indicating similar protease specificity. Two potential glycosylation sites (amino acids NIS shown as a triangle at position 247–249 and amino acids SARP shown as a circle at position 355–358) were found in mouse PCI, while human PCI contains five potential glycosylation sites (corresponding sequences are shown as circles and triangles). On the protein level there is a 63% homology between mouse and human PCI.

trations being present in pancreas. In the mouse, however, PCI expression was exclusively detected in the reproductive system (testis, seminal vesicle, ovary). No PCI mRNA was found in mouse liver, suggesting that in the mouse PCI is not a plasma protein.

From the tissue distribution, it follows that PCI might fulfill different biological functions in mice and humans, although the proteins are highly homologous.

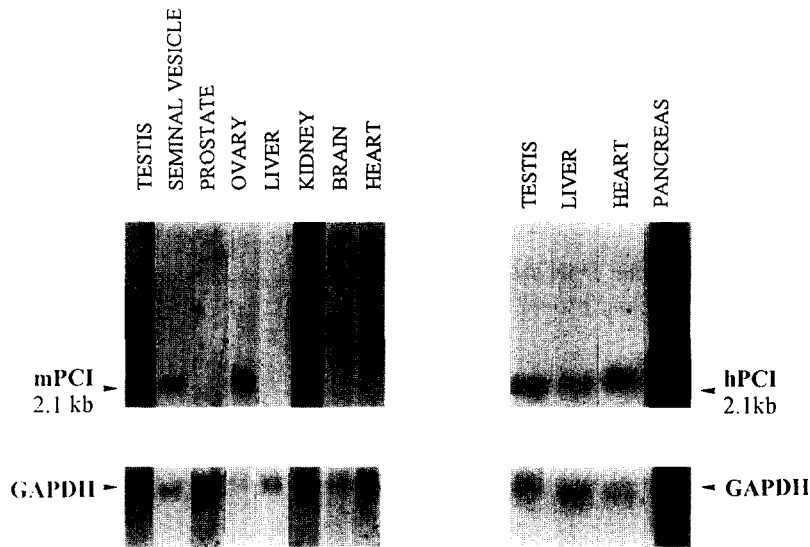


Fig. 2. Tissue-specific expression. Mouse PCI (mPCI) compared to human PCI(hPCI).

References

- Ecke, S., Geiger, M., Resch, I., Jerabek, I., Stingl, L., Masier, M., and Binder, B.R. (1992) *J. Biol. Chem.* 267: 7048.
- Geiger, M., Huber, K., Wojta, J., Stingl, L., España, F., Griffin, J.H., and Binder, B.R. (1989) *Blood* 74: 727.
- Marlar, R.A., and Griffin, J.H. (1980) *J. Clin. Invest.* 66: 1186.
- Meijers, J.C.M., and Chung, D.W. (1991) *J. Biol. Chem.* 266: 15028.
- Meijers, J.C.M., Kanters, D.H.A.J., Vlooswijk, R.A.A., van Erp, H.E., Helsing, M., and Bouma, B.N. (1988) *Biochemistry* 27: 4231.
- Suzuki, K., Nishioka, J., Kusumoto, H., and Hashimoto, S. (1984) *J. Biochem. (Tokyo)* 95: 187.
- Suzuki, K., Deyashiki, Y., Nishioka, J., Kurachi, K., Akira, M., Yamamoto, S., and Hashimoto, S. (1987) *J. Biol. Chem.* 262: 611.
- Suzuki, K., Deyashiki, Y., Nishioka, J. and Toma, K. (1989) *Thromb. Haemostasis* 61: 337.
- Zheng, X.L., Geiger, M., Ecke, S., Bielek, E., Donner P., Eberspächer, U., Schleuning, W.-D. and Binder, B.R. (1994) *Am. J. Physiol.* 267 (Cell Physiol. 36): C466.