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Genomic organisation of the mouse gene encoding endothelin-converting enzyme-1 (ECE-1) and mRNA expression of ECE-1 isoforms in murine tissues

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Abstract

Mouse knockout-models have previously revealed important biological functions of endothelin-converting enzyme-1 (ECE-1) in normal cardiac and craniofacial development. Since human ECE-1 is expressed in various isoforms, termed a, b, c, and d, expression of which is controlled by alternative promoters, we postulated that corresponding isoforms may also be transcribed from the murine *Ece1* gene. By comparative sequence analysis using exon-specific sequences of human and rat ECE-1 we have resolved the complete exon–intron structure of the murine *Ece1* locus on chromosome 4. The murine *Ece1* gene comprises 23 exons distributed over 100 kb of genomic DNA and was found to be structurally highly conserved when compared to the human *ECE1* gene. As with the human gene, the exons containing isoform-specific sequences were localised in the 5′ terminal region of the murine *Ece1* gene. Using specific sense primers, isoform-specific expression of murine ECE-1 mRNA in various mouse tissues was confirmed by RT-PCR. Using real-time PCR we demonstrated that ECE-1c was the most abundantly expressed isoform in most tissues, except for heart and aorta displaying a more even isoform distribution. We detected an additional isoform-specific exon, designated c2, which was apparently constitutively spliced and expressed only as minor fraction of ECE-1c transcripts. Our results provide evidence of structural conservation of mammalian genes encoding ECE-1 and will facilitate a more refined analysis of ECE-1 mRNA expression in the mouse model organism.

Keywords: Metalloprotease; Exon–intron structure; Real-time PCR; Splice variant

1. Introduction

Endothelin-converting enzymes (ECEs) belong to the neprilysin family of zinc-binding metalloproteases (Turner et al., 2001) and are considered as a main source of biologically active endothelin peptides. ECE-1 represents a transmembrane type II metalloprotease and forms a dimer stabilised by a disulfide-bond linking the larger extracytoplasmic domains of the protein (Shimada et al., 1996). In humans, ECE-1 was reported to be expressed not only in the endothelium, its primary site of constitutive expression, but also in certain other epithelial and mesenchymal cell types which is in agreement with the known pleiotropic actions of endothelin peptides (Korth et al., 1999; Kedzierski and Yanagisawa, 2001). Cloning of ECE-1 cDNA has been reported in human, bovine and rat species showing significant interspecies conservation (Schmidt et al., 1994; Yorimitsu et al., 1995; Shimada et al., 1995a, 1994; Xu et al., 1994; Ikura et al., 1994). The developmental significance of ECE-1 was studied by generation of mice deficient in ECE-1 (*Ece1*$^{-/-}$ mice) displaying a complex phenotype which can be summarised as a composite of the phenotypes previously observed in mice deficient of ET-1 or ET_A receptor and in mice deficient of ET-3 or ET_B receptor, respectively, including

Abbreviations: bp, base pair; Ct, cycle threshold; dCt, delta Ct ($Ct_{target\ gene} - Ct_{endogenous\ control\ gene}$); ddCt, delta delta Ct ($dCt_{target\ gene\ x} - dCt_{target\ gene\ y}$); ECE, endothelin-converting enzyme; ET, endothelin; ET_A, endothelin receptor type A; ET_B, endothelin receptor type B; kb, kilobase; PCR, polymerase chain reaction; RT, reverse transcription.

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craniofacial, neuronal and cardiovascular malformations (Yanagisawa et al., 1998). ECE-1 appears to play a similar developmental role in humans since a patient with a mutation in the *ECE1* gene showed abnormalities closely resembling the phenotype of *Ece1*$^{-/-}$ mice (Hofstra et al., 1999). More recently, investigations in *Ece1*$^{-/-}$ mice provided evidence of a protective role for ECE-1 in Alzheimer's disease since ECE-1 was demonstrated to process β-amyloid thereby reducing amyloid deposition in the murine brain (Eckman et al., 2003).

Analysis of transcripts generated from the human *ECE1* gene has revealed expression of ECE-1 mRNA isoforms, designated ECE-1a (formerly also termed ECE-1 beta), ECE-1b (formerly also termed ECE-1 alpha), ECE-1c, and ECE-1d, respectively. These isoforms differ only in their 5′ terminal sequences, resulting in variant N-termini (Schweizer et al., 1997; Valdenaire et al., 1999). In the human *ECE1* gene, the isoform-specific exons are distributed over 66 kb in the 5′ terminal region (Fig. 1), with the c-specific exon located at the extreme 5′ end (Funke-Kaiser et al., 2000), followed in 3′ direction by exons 1b and 1d, respectively (Valdenaire et al., 1995). Exon 1d is immediately adjacent to exon 2 that is shared by isoforms b, c, and d, respectively. Exon 1a is located most 3′ of all isoform-specific exons and is immediately adjacent to exon 3 which is part of all ECE-1 isoforms. We and others have previously shown that expression of human ECE-1 isoforms is regulated by alternative promoters located immediately upstream of each isoform-specific exon (Orzechowski et al., 1997; Valdenaire et al., 1999; Funke-Kaiser et al., 2000). Specific induction of ECE-1a transcription, but not of the other isoforms, in human endothelial cells subsequent to activation of protein kinase C has recently been demonstrated by our group (Orzechowski et al., 2001).

Analysis of rat and bovine ECE-1 transcripts revealed isoform expression which corresponded to human ECE-1 isoform diversity (Shimada et al., 1995b; Valdenaire et al., 1999; Meidan et al., 2005). It is has not been investigated in detail whether the complexity of ECE-1 isoform expression as demonstrated in humans may also exist in the mouse nor has the complete structure of the gene encoding ECE-1 been explored in mammalian species other than human. Because of the outstanding significance of the mouse as a model organism of human disease and embryonic development the principal aim of this study was to resolve the complete exon–intron structure of the murine *Ece1* gene, to identify putative murine ECE-1 isoforms and to study their mRNA expression in mouse tissues.

2. Materials and methods

2.1. Computational analysis the exon–intron structure of the murine Ece1 gene

The general exon–intron structure of the murine *Ece1* gene on chromosome 4 (subregion D3) was analysed by sequence alignment of genomic murine ECE-1 nucleotide sequence (AL807764) with ECE-1 cDNA sequence (NM_199307) using the bl2seq algorithm. We used the nucleotide–nucleotide BLAST (blastn) algorithm to screen the nucleotide database applying human and rat isoform-specific ECE-1 sequences as "probes" [ECE-1c: X98272 (human), D29683 (rat); ECE-1b:

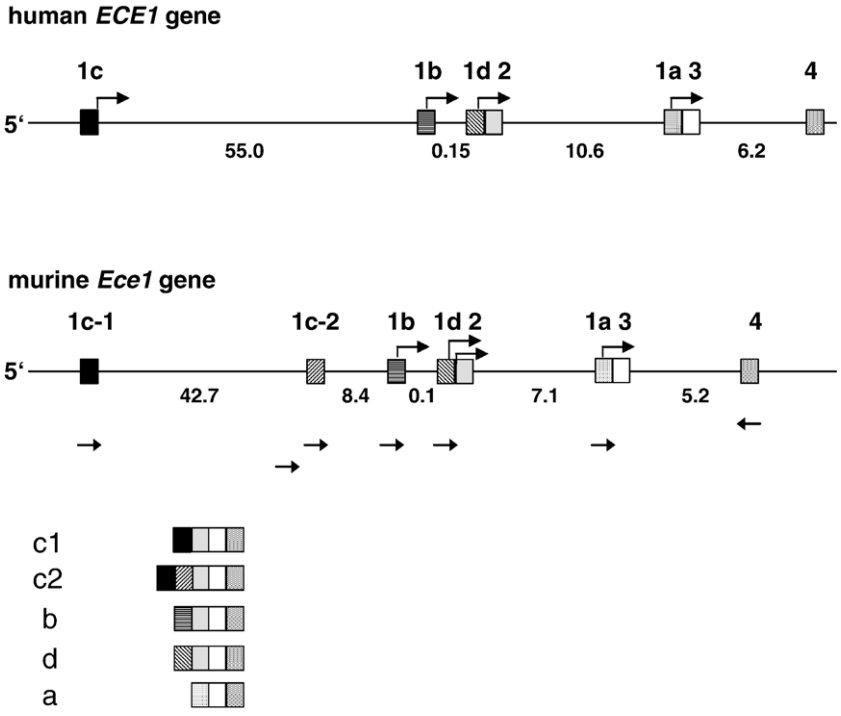

Fig. 1. Structural conservation of human and murine genes encoding ECE-1. The 5′ terminal regions of human *ECE1* and murine *Ece1* genes are depicted. Isoform-specific exons are indicated and depicted as boxes. Angled arrows indicate putative translational start codons. Arrows below the murine gene indicate positions of isoform-specific sense primers and of the common antisense primer (exon 4), respectively. 5′ ends of the isoform-specific transcripts are shown at the bottom. Numbers below the graphs indicate intron sizes in kb.