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Tissue-specific expression pattern of human endothelial lipase in transgenic mice

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Abstract

Endothelial lipase (EL), a new member of the triacylglycerol lipase gene family, is a key enzyme in HDL metabolism. The EL expression pattern in humans was reported to be unique and complementary to that documented for lipoprotein lipase. The regulatory elements responsible for the tissue-specific EL expression are not identified yet. In order to confine these sequences to a defined region of the EL promoter, we analyzed EL mRNA expression in human EL transgenic mice expressing EL under the control of the endogenous human promoter. We identified small intestine, mammary gland, adipose tissue and the adrenal gland as previously unknown tissues to express EL. Our data demonstrate that regulatory elements within 11.4 kb of 5′ and 9.9 kb of 3′ human EL flanking region promote the expression of EL in small intestine, ovary, testis, mammary gland, brain, lung, aorta, adipose tissue and the adrenals, whereas regulatory sequences located between 27.4 and 11.4 kb of 5′ or 9.9 and 48.7 kb of 3′ human EL flanking region seem to be responsible for kidney-specific EL expression.

Keywords: Endothelial lipase; Transgenic mouse; Endogenous promoter; mRNA expression; Real-time PCR

1. Introduction

Endothelial lipase (EL) is a key enzyme in HDL metabolism. Adenoviral and transgenic overexpression of human EL in mice resulted in significantly reduced HDL-cholesterol (HDL-C) and phospholipid levels due to increased HDL catabolism [1–4]. Conversely, antibody inhibition of murine EL activity [5] and gene specific knockout of murine EL [3,6] resulted in significantly increased HDL-C and phospholipid levels. Several EL gene variants were shown to be associated with high HDL-C levels in humans [6–11].

The human EL gene was cloned and characterized 1999 independently by two different groups [1,12]. EL's amino acid sequence revealed 45% homology with lipoprotein lipase (LPL) and 40% with hepatic lipase, indicating that this gene was a new member of the triacylglycerol lipase gene family [1,12]. In contrast to other family members, EL is synthesized by endothelial cells and thus has been termed endothelial lipase (encoded by the LIPG gene) [1,12]. In addition to endothelial cells, EL expression was detected in the human HepG2 and mouse Hepa 1-line, the human THP-1 and mouse RAW 294.7 macrophage cell lines and mouse yolk sac cells [1,12]. In the adult human, EL expression was described to be complementary to that documented for LPL. LPL is expressed in muscle tissues, adipose tissue, mammary gland, brain and macrophages, whereas EL was shown to be expressed in placenta, liver, lung, kidney, testis, ovary and thyroid [1,12]. The regulatory elements responsible for the tissue-specific EL expression are currently not identified. In the present study, we were able to confine these regulatory elements to a defined region of the human EL promoter by analyzing human EL transgenic mice expressing EL under the control of the endogenous human promoter. Furthermore, we identified human EL mRNA in several tissues that were previously not known to express EL.

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2. Materials and methods

2.1. Generation of transgenic mice

A P1 clone containing 11.4 kb of the 5′ flanking region of human EL, all 10 exons and introns of EL and 9.9 kb of the 3′ flanking region as determined by sequencing was purchased from Incyte Genomics Inc. (Palo Alto, California, USA). The P1 clone DNA was purified using the Geneclean II kit (Bio 101, Inc.) according to the manufacturer's protocol and eluted in injection buffer (10 mM Tris/0.1 mM EDTA, pH 7.5). Transgenic mice were produced by microinjection of the purified DNA into the male pronucleus of fertilized mouse oocytes. Fertilized oocytes were harvested from superovulated B6SJLF1/J females mated with B6SJLF1/J males. Founder mice were screened for transgene integration by PCR amplification using oligonucleotides that were specific for the transgene (forward primer: 5′-TGCGGCCGCTAATACGACTCACTATAGG-3′, reverse primer: 5′-AGTTCACAAAAGCCAAACCACCTGGTTG-3′). Of 52 newborn mice, five mice harboured the human EL transgene. Founder animals were crossed with C57BL/6J mice, and genomic DNA was extracted from the tails of the offspring to determine the copy number of the transgene by performing real-time PCR analysis. The mouse line with the highest copy number was used for further experiments. The protocols for mice have been approved by the University of Pennsylvania Animal Care and Use Committees (IACUC) and meet their standard guidelines.

2.2. Determination of the transgene copy number

Mouse tail DNA was extracted using the High Pure PCR Template Preparation Kit (Roche) according to the manufacturer's protocol. Transgene copy number was determined in F1 generation mice by real-time PCR (ABI Prism 7700 Sequence Detector System, PE Applied Biosystems) using the absolute standard curve method. Human genomic DNA was used to generate a standard curve. The primer/probe combination for human EL was confirmed to be specific for the targeted gene (human EL forward primer: 5′-ACAACCTGTGGAAGGAGTTTCG-3′, reverse primer: 5′-CGGATGCGCCTGATATTCA-3′, TaqMan probe: 5′-FAM-CTGTCTCAACCCCGCAACCCCGGA-TAMRA-3′).

2.3. Quantitation of human EL mRNA expression levels

Total RNA was extracted from tissues of 8 week-old F2 generation mice after a 4 h fast using the RNeasy Kit (Qiagen) according to the manufacturer's protocol. RNA (0.5 μg) were digested with DNase I Amplification Grade (Invitrogen) and subsequently transcribed into cDNA using Invitrogen's SuperScript II First Strand Synthesis System. The level of human EL mRNA expression was determined by real-time PCR (ABI Prism 7700 Sequence Detector System, PE Applied Biosystems) using mouse GAPDH (forward primer: 5′-GCCTCGTCCCGTAGACAAAA-3′, reverse primer: 5′-TGGCAACAATCTCAACTTTGC-3′, TaqMan probe: 5′-FAM-CAGGCGCCCAATACGGCCAA-TAMRA-3′) as a housekeeping gene. Gene expression was determined in three male and three female EL transgenic mice. Primer/probe combinations for real-time PCR were confirmed to be specific for the targeted genes. All real-time PCR samples were run in duplicates and the results were highly reproducible. The comparative CT method ($2^{-\Delta\Delta C_T}$) was used to quantitate mRNA expression.

2.4. Statistical analysis

Data are expressed as the mean ± S.E.M. Analysis of the data was performed using the Student's *t*-test. Statistical significance for all comparisons was assigned at $p < 0.05$.

3. Results and discussion

Five independent lines of transgenic mice were established with 1–5 copies of a P1 clone containing the whole human EL genomic sequence, 11.4 kb of the 5′ flanking and 9.9 kb of the 3′ flanking region of EL. The mouse line with the highest copy number (5 copies/genome) was used for the study described in this paper. Littermates negative for human EL were used as controls.

In an effort to characterize the expression pattern of human EL mRNA in the transgenic mice, a real-time PCR was established for human EL as the target and mouse GAPDH as a housekeeping gene. An initial validation experiment demonstrated that the efficiency of the target and the reference amplification is approximately equal (slope (hEL) − slope (mGAPDH) equalled 0.08). Therefore, the

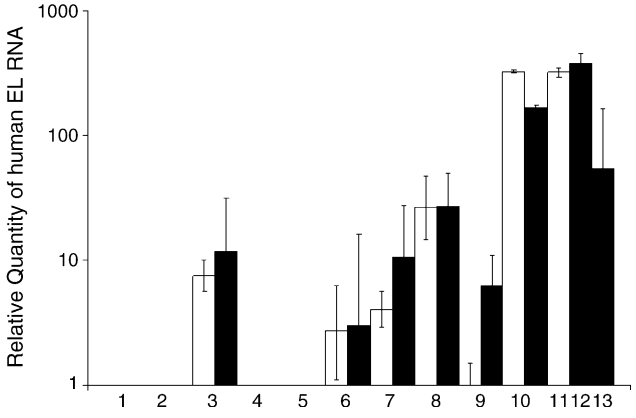

Fig. 1. Human EL mRNA expression in EL transgenic mice. Human EL mRNA expression is normalized to mouse GAPDH as an endogenous reference. EL mRNA expression levels are shown relative to EL expression in the male adrenal gland, the organ with the lowest EL expression level. White bars depict male, black bars female animals (three animals/group). Data are presented as the mean ± S.E.M. (1) Skeletal muscle, (2) heart, (3) lung, (4) liver, (5) kidney, (6) adipose tissue, (7) aorta, (8) brain, (9) adrenal gland, (10) jejunum, (11) testis, (12) ovary, (13) mammary gland.