Alternative Promoters Determine Tissue-specific Expression of the Human Microsomal Epoxide Hydrolase Gene (EPHX1)

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ABSTRACT

Microsomal epoxide hydrolase (EPHX1) catalyzes hydration reactions that determine the cellular disposition of reactive epoxide derivatives derived from xenobiotic exposures. Whereas the previously defined human EPHX1 exon 1 sequence, E1, is derived from a gene promoter immediately proximal to exon 2 of the EPHX1 coding region. Remarkably, the E1 promoter directs expression only in the liver. We have identified an alternative EPHX1 promoter that is localized ~18.5 kb upstream of exon 2. Northern hybridizations demonstrated that the E1b promoter functions as the primary driver of EPHX1 expression in human extrahepatic tissues. Using a rolling circle amplification-RACE technique, together with EST database analyses, we have identified 2 distinct EPHX1 transcripts in extrahepatic tissues and cell lines that are derived from the E1b promoter region. We term the transcripts E1-b and E1-b'. The E1-b transcript includes a noncoding 46 bp exon 1 in its 5'-untranslated region. In contrast, the length of the E1-b' 5'-UTR is extended, and includes 2 upstream AUG codons together with high GC content. Analyses of the human E1b gene promoter region indicated the presence of multiple transposable elements, including Alu repetitive elements of the AluSp, AluSx, AluJo and AluYa5 classifications. The AluYa5 elements are relatively recent in evolutionary history, and their presence in the EPHX1 promoter was associated with significant reduction in EPHX1 transcriptional activity following transfection experiments conducted in transformed human cell lines derived from multiple tissues, including kidney 293A, lung A549, hepatoma HepG2, and breast cancer MCF7. Further investigations are underway to assess the regulatory features of this promoter that direct its transcriptional activity in human tissues.

INTRODUCTION

Figure 1.

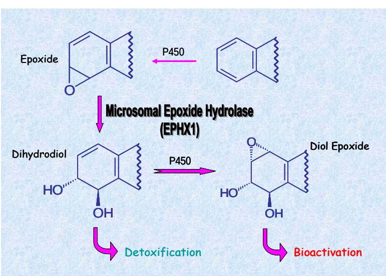

Figure 1. Biotransformation pathways for the carcinogenic polyaromatic hydrocarbons.

Figure 2.

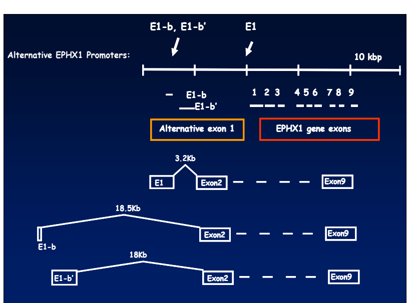

Figure 2. Basic structural organization of the human EPHX1 gene locus.

RESULTS

Figure 3.

Northern Blot demonstrating liver specific expression of the EPHX1 E1 transcript and multi-tissue expression of the E1-b transcript.

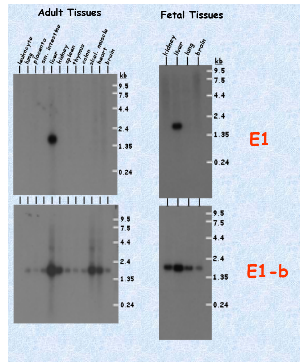

Figure 4.

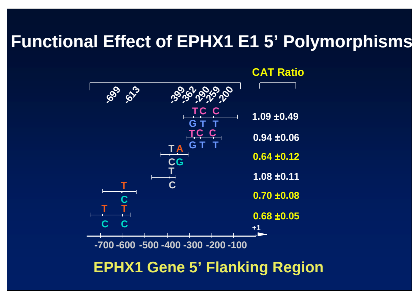

Figure 4. Functional effect of EPHX1 E1 5' polymorphisms. The proximal EPHX1 5' flanking region is depicted along with sites of polymorphism identified at –699, –613, –399, –362, –290, –259 and –200. The CAT ratios were derived using the normalized chloramphenicol acetyl transsferase (CAT) reporter expression. Highlighted values are statistically significant at p< 0.05.

Figure 5.

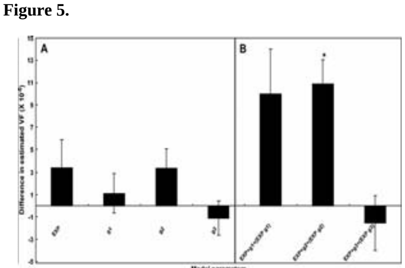

Figure 5. Correlation of human sensitivity to butadiene exposures and polymorphisms in the E1 5'-flanking promoter region. (A) Estimated change in HPRT mutant frequency (VF) from background (baseline) level, computed using the general linear model, as it relates to butadiene exposure (Exp) alone, polymorphisms in g1, g2, and g3 alone (corresponding to the presence of the homozygous or heterozygous variant alleles in the -200 linkage, -600 linkage, and the -399 site, respectively). **(B)** Estimated change relates to the interaction between exposure (Exp) and the presence of the polymorphisms in g1, g2, and g3.

RESULTS (continued)

Figure 6.

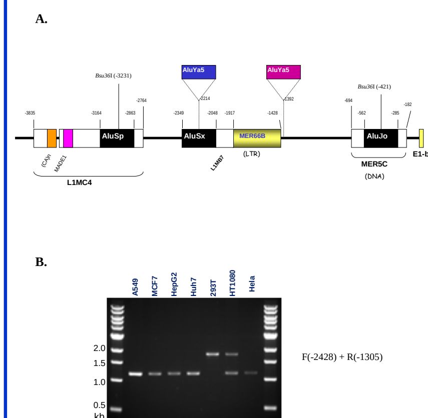

Figure 6. Identification of Alu insertion polymorphisms in the proximal upstream region of the E1-b EPHX1 promoter. (A) Alu insertion/deletion polymorphisms in the 5'-flanking region of the E1-b promoter. Also shown are the transposable element (TE) cassettes imbedded in the upstream promoter region. **(B)** PCR analysis of the E1-b 5'-flanking region in A549, MCF7, HepG2, Huh7, 293T, HT1080 and Hela cells. HT1080 is heterozygous; 293T is homozygous 2xAlu; others are homozygous without the Alu elements.

Table 1.

Relative promoter activities

Cell types	Haplotypes No Alu	Haplotypes 2xAlu
A549	100 ± 7	61 ± 9
HepG2	100 ± 7	71 ± 10
MCF7	100 ± 9	89 ± 12
293A	100 ± 7	77 ± 9

Table 1. Analysis of promoter activities of polymorphic E1-b 5'-flanking region. Luciferase-based promoter activities were analyzed by cloning the E1-b 5'-flanking DNA region (-2763/+6) for different haplotypes into the pGL3-basic vector. The data shown are derived from five separate experiments, 2xAlu showing a statistically significant decreased promoter activity (p<0.05).

ACKNOWLEDGEMENTS

We thank Dr. Chris Hassett for his initial insights and contributions to this project and Denise Weyant for expert technical assistance.

RESULTS (continued)

Figure 7.

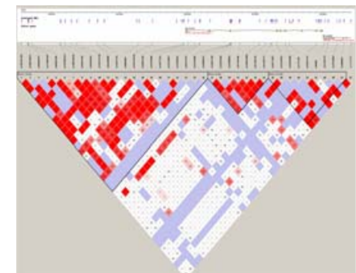

Figure 7. Haplotype block structure of a 52 kb region spanning EPHX1 and the extended promoter regions in European Americans. HapBlocks are indicated within solid blank-lined triangles. Polymorphisms in the E1 promoter region and the E1-b promoter are in linkage disequilibrium (LD) with each other but not with coding region polymorphisms described previously in exons 3 and 4.

SUMMARY AND CONCLUSIONS

- ❑ Alternative promoters drive EPHX1 expression according to tissue specific programs. The liver, as an essential detoxification organ, uses both E1 and E1-b.
- ❑ Polymorphisms within the E1 promoter region likely modulate the transcription of the EPHX1 gene, and appear to contribute to individual sensitivities to butadiene exposure.
- ❑ Insertion of Alu elements within the E1-b promoter region results in decreased transcriptional activity, in *vitro*.
- ❑ Haplotype analyses indicate that the polymorphisms in the two distinct promoter regions reside within the same HapBlock.

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