Supplementary Materials and Methods

SDS-Gelatin and In Situ Zymography

Briefly, 20–40 g of lysate protein was separated by SDS (10%)-PAGE copolymerized with 0.1% gelatin (Gibco, Carlsbad, CA) or purchased commercially (Invitrogen). Gels were washed in 2.5% Triton X-100 and then incubated overnight in developing buffer. After Coomassie blue staining, gels were destained, and the amount of MMP activity detected as clear bands was analyzed by densitometry. The intermediately active form (66 kilodaltons) of human MMP-2 (10–20 ng; Calbiochem, Gibbstown, NJ) was used as a control.

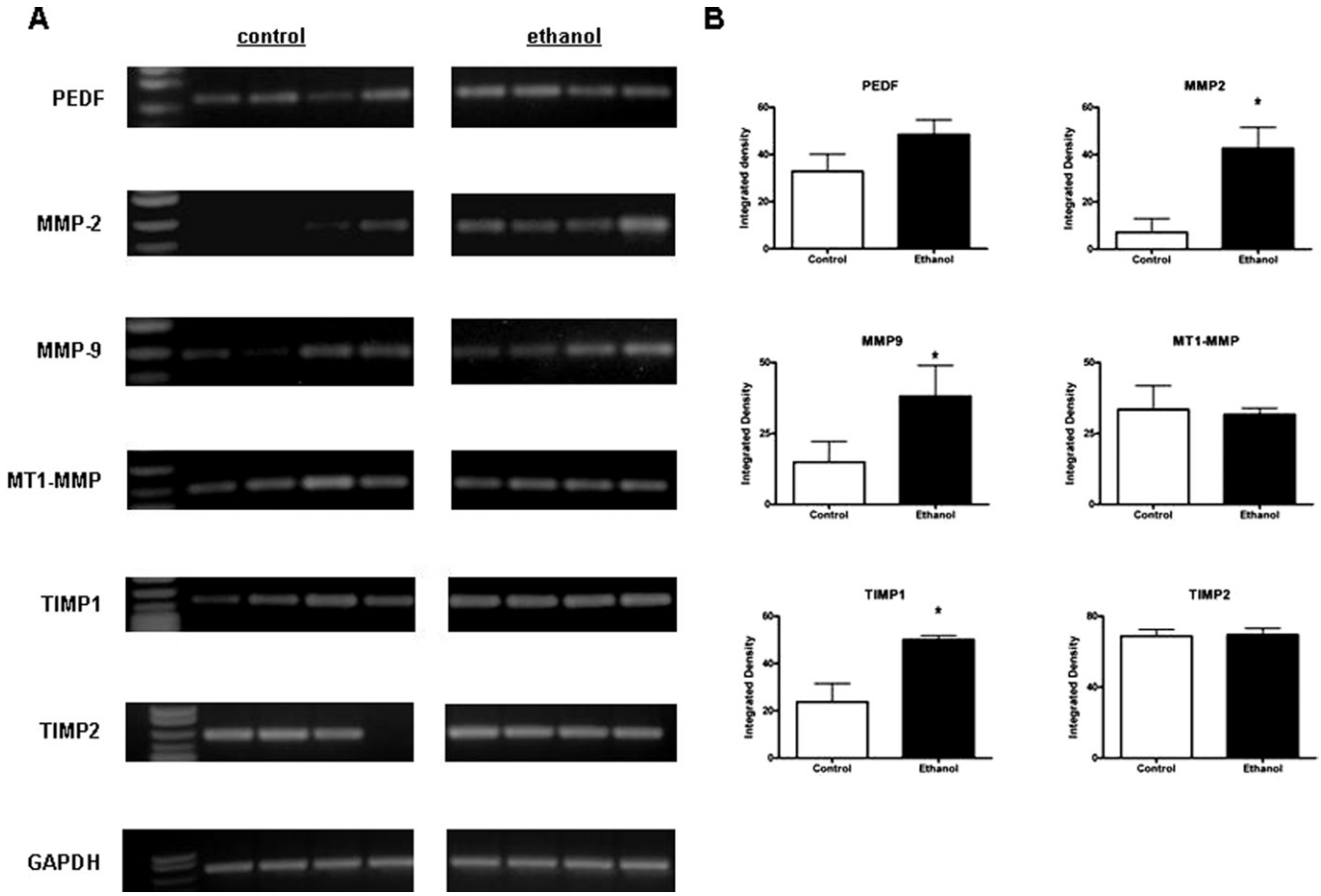
To localize areas associated with MMP-2/9 (gelatinase) activity, in situ zymography was performed using a modified protocol from one described.[1] Briefly, 10-mol/L frozen liver sections were incubated for 60 minutes at 37 C in the presence and absence of APMA-containing buffer. Sections were then covered with a solution containing equal amounts of Oregon Green 488-conjugated gelatin (Molecular Probes, Eugene, OR) and 1% low melting temperature agarose. Sections were incubated for 12 hours, and gelatinolytic activity was detected as areas of clearing in the fluorescent substrate.

Reverse-Transcription Polymerase Chain Reaction Procedures

Hepatic gene expression of PEDF, MMP-2/9, MT1-MMP, and TIMP-1/2 was performed by reverse-transcription polymerase chain reaction (RT-PCR) using total RNA from ethanol-fed and control mice livers. GAPDH served as loading control. RNA was extracted using RNeasy (Qiagen) purification kit. Complementary DNA (cDNA) synthesis was performed as described.[2] PCR primers are listed in supplementary Table 1. PCR was performed in 50 L PCR buffer (45 L Platinum Blue PCR SuperMix [Invitrogen]), 200 nmol/L each primer, 2 L cDNA). After a 30-second denaturation step at 94 C, the reaction proceeded in 35 cycles of 30 seconds at 94 C, 30 seconds at 55 C, 30 seconds at 72 C, followed by 10 minutes at 72 C. Ten L of each PCR product were separated on a 1% agarose gel and stained with ethidium bromide.

References

1. Frederiks WM, Mook OR. Metabolic mapping of proteinase activity with emphasis on in situ zymography of gelatinases: review and protocols. J Histochem Cytochem 2004;52:711–722.
2. Notari L, Baladron V, Aroca-Aguilar JD, et al. Identification of lipase-linked cell membrane receptor for pigment epithelium-derived factor. J Biol Chem 2006;281:38022–38037.



Supplementary Figure 1. Semiquantitative RT-PCR analysis reveals stable hepatic PEDF gene expression, whereas MMP-2 and -9 expression are increased with ethanol feeding. (*A*) Ethanol-exposed mouse livers resulted in unchanged PEDF expression compared with control tissues (P = NS). MMP-2 and -9 expression levels were both significantly increased by ethanol feeding (*$P < .05$). TIMP1 expression was increased (*$P < .05$), whereas no significant differences in TIMP2 or MT1-MMP expression levels were detected. (*B*) Corresponding densitometry.

Supplemental Table 1. List of primers used for RT-PCR analysis

	Forward Primer	Reverse Primer
PEDF	TGGCCACGCTGAGTGGAGACAGCAG	GCATGCTTGGTACTTTATCTAC
MMP2	GTTCTGGAGATACAATGAAGTG	CACCCTCTTAAATCTGAAGTC
MMP9	GTACCAAGACAAAGCCTATTTCTG	GCTGATTGACTAAAGTAGCTGG
MT1-MMP	CTTCAAAGGAGATAAGCACTG	GTTTCCCTTGTAGAAGTATGTG
TIMP1	CAGAAATCAACGAGACCACC	GGGATAGATAAACAGGGAAACAC
TIMP2	ATGCAGACGTAGTGATCAGAG	AGATGTAGCAAGGGATCATGG