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Human Chromosomal Localization, Tissue/Tumor Expression, and Regulatory Function of the *ets* Family Gene *EHF*

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***Ets* factors are members of an ancient multigene family of transcription factors including oncoproteins and possibly tumor suppressors. We previously characterized a novel divergent *ets* gene, *Ehf* (*ets homologous factor*) in mice. Here we report the cDNA sequence, chromosomal location, and tissue/tumor expression patterns of the human *EHF* gene and the regulatory activity of the EHF protein. *EHF* maps to 11p12, which is deleted in many prostate, breast, and lung carcinomas and is a hot spot for inherited deletion- or amplification-associated developmental defects. *EHF* is differentially expressed in normal tissues and carcinomas and between tumor stages and is most highly expressed in the organs known to form carcinomas upon 11p12 deletion. EHF protein represses the ETS-2 induced activity of both stromelysin-1 and collagenase-1 promoters. These data suggest that *EHF* may contribute to human development and carcinogenesis and is a candidate for the 11p12 tumor suppressor gene.**

Ets family transcription factors are identified by a conserved DNA-binding domain, the "ETS domain" (1, 2). Since the first *ets* protein was discovered as part of the hybrid avian leukemia virus E26 (3), over 30 additional *ets* genes have been detected in metazoans (4). Interest in *ets* factors has grown due to their ability to differentially regulate gene transcription and induce cancer. They target promoters by forming complexes with unrelated transcription factors and by affecting their regulatory activity (5, 6). Most *ets* proteins are transactivators and are either known oncoproteins, many of which are activated by chromosomal translocations in human malignancies (7–17), or are upregulated in proliferating cells (8, 11, 18–25). A recently discovered *ets* gene, *ELF5*, is proposed to encode a tumor-suppressing transactivator. It maps to a tumor suppressor locus for carcinomas of the same organs in which it is most highly expressed, loses its expression in such tumors, and may transactivate the maspin tumor suppressor gene (26). Other *ets* proteins are transcription repressors, such as human NERF-1 (27) and ERF (28–30). *ERF* maps to a chromosomal locus associated with leukemogenesis and oncogenesis (30) and has been proposed to act as a tumor suppressor rather than an oncoprotein (28, 30). Finally, some *ets* factors such as ERG and ETS-1 can act not only as transactivators but also as repressors, depending on the composition of the target promoter and its interacting transcription factor complexes (31, 32). Therefore they may exhibit both oncogenic and tumor suppressing properties in different cells. Many *ets* factors thus appear to contribute to cancer through altered transactivator or repressor activity.

Ets factors may cause cancer in part by regulating genes for matrix metalloproteinases (MMPs) influencing not only normal growth and development but also tumor invasion, metastasis and angiogenesis (33–35). MMP promoters contain multiple *ets* binding site (EBS) motifs, some adjacent to AP-1 motifs, and their number and organization within the promoter affects

The nucleotide sequence has been deposited in GenBank under Accession No. AF170583.

Abbreviations used: aa, amino acid(s); nt, nucleotide(s); bp, base pair(s); kb, kilobase pair(s); ORF, open reading frame; UT, untranslated; cDNA, complementary DNA; EBS, ets-binding site; MMP, matrix metalloproteinase; ECM, extracellular matrix.

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the specificity of MMP regulation by *ets* factors. We and others have shown that in transient transfection assays, ETS-1, ETS-2 and E1AF activate the stromelysin-1, collagenase-1, and 92 kDa MMP promoters (36–38), while ERG activates the collagenase-1 promoter but strongly represses the stromelysin-1 promoter, via the formation of different multiprotein complexes between the *ets* factors, Fos, Jun and the coactivator p300/CBP (31, 39, 40). The importance of such EBS motifs and their *ets* factor interactions in cancer is supported by our recent finding that a single nucleotide polymorphism (SNP) in the collagenase promoter increases collagenase expression in cancer cells by creating an additional novel EBS next to an AP-1 site (41). This is the first example of MMP expression in cancer affected by *ets*-binding site variation, and supports the concept that *ets* factors and their variable promoter and transcription factor interactions regulate ECM degradation and cancer progression.

Previously, we characterized a new and highly divergent member of the *ets* gene family, "*ets* homologous factor" (*Ehf*) from mice (42). Because *Ehf* cDNA was isolated from early-stage pituitary somatotroph tumors, this suggested a possible role for *Ehf* in regulating cellular proliferation and solid tumor development (42). Here we have characterized the chromosomal location and normal and tumor tissue expression of *Ehf*'s human orthologue, *EHF*, and examined the regulatory effect of the EHF factor on the collagenase-1 and stromelysin-1 promoters. These data suggest *EHF* may play a role in the development of major carcinomas in humans.

MATERIALS AND METHODS

Human EHF cDNA isolation. Searching GenBank with mouse *Ehf* cDNA sequence identified a human Expressed Sequence Tag (EST) cDNA clone (Accession No. AA149006) that strongly matched the 3′ end of *Ehf*'s ORF and its 3′ UT region. This 1.6-kb cDNA clone was obtained from Genome Systems Inc. and its sequence verified and extended by fluorescence-tagged dideoxy-sequencing on an Applied Biosystems model 373A automated sequencer. Additional *EHF* sequence was obtained from "Marathon Ready" human prostate cDNA (Clontech) by 5′ rapid amplification of cDNA ends (5′ RACE), subcloning into TA cloning vector pCR2.1 (Invitrogen) and sequencing as above. A ^{32}P-α-dCTP radiolabeled, PCR-amplified probe from the 3′ UT region of human *EHF* was hybridized to 10 μg of Southern-blotted human spermatocyte and mouse tail genomic DNAs digested with 5 U/μg DNA of *Eco*RI (or *Bam*HI or *Hin*dIII, not shown) to confirm *EHF* was orthologous to mouse *Ehf*. The positive control mouse *Ehf* probe was a similarly radiolabeled PCR-amplified adjacent ORF segment. Overnight to several-day autoradiographs with an intensifier screen (Fisher) were made to detect the single-copy gene signal.

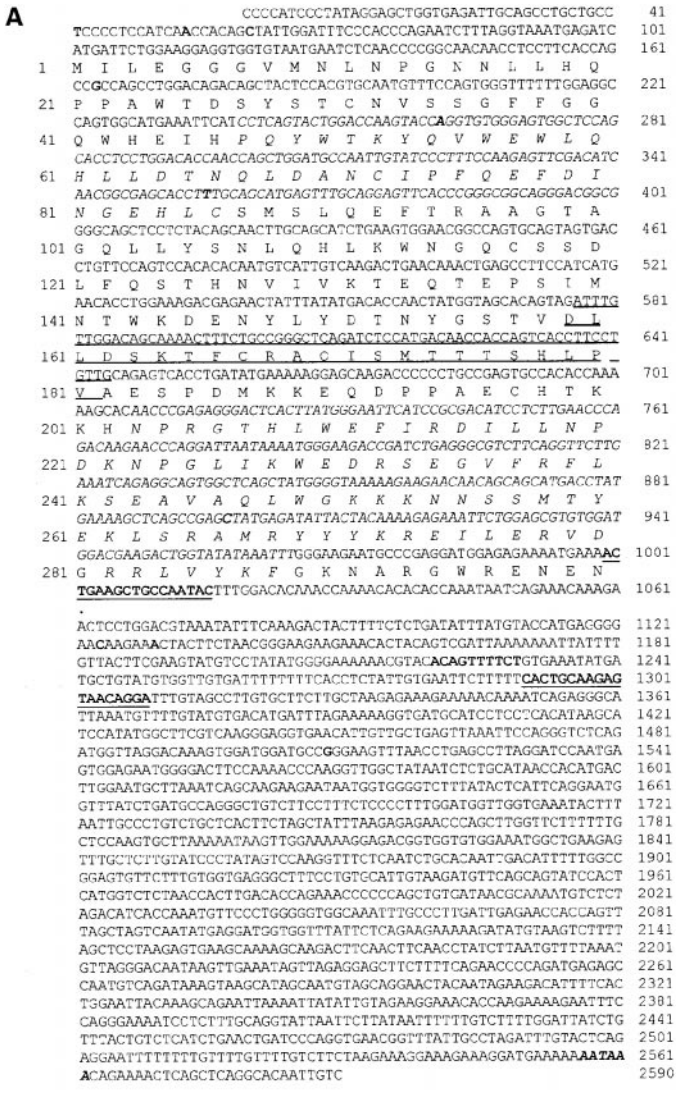
Human chromosomal mapping of EHF. The 1.6-kb human *EHF* 3′ UT region cDNA clone was used to prepare a probe for fluorescence *in situ* hybridization (FISH) chromosomal mapping on high-resolution chromosome spreads. Clone labeling and FISH mapping was performed by SeeDNA Biotech Inc. as previously described (43, 44), using cDNA probe biotinylation with dATP at 15°C for 1 h. (GIBCO BRL BioNick labeling kit). Briefly, lymphocytes isolated from human blood were cultured 68–72 h at 37°C in α-minimal essential medium (α-MEM) with 10% fetal calf serum (FCS) and phytohemagglutinin, treated with bromodeoxyuridine (0.18 mg/ml, Sigma) to synchronize the cells, washed three times with serum-free medium to release the block, and recultured 6 h at 37°C in α-MEM with thymidine (2.5 μg/ml, Sigma). Cells were harvested and chromosome slides made by standard hypotonic treatment, fixation and air-drying. Slides were baked 1 h at 55°C, RNAse treated, denatured 2 min at 70°C in 70% formamide with 2× SSC, and ethanol dehydrated. Probes were denatured 5 min at 75°C in hybridization mix with 50% formamide and 10% dextran sulfate and loaded on the slides. After overnight hybridization, slides were washed, detected and signal-amplified. FISH signals and DAPI banding patterns on the high-resolution chromosomes were recorded in separate photos, with signal superimposition used to assign the FISH signals to the chromosomal bands.

RNA blot analysis of EHF expression in human normal and tumor tissues. A human multi-tissue mRNA dot blot (Clontech) with poly A$^+$ RNA from adult and fetal human tissues in 89–514 ng quantities per dot (normalized for equal expression of multiple housekeeping genes), and a human multi-tumor dot blot (Biochain Institute, Inc.) containing 5 μg of total RNA per dot from multiple human tumor types and stages, were hybridized with the radiolabeled *EHF* 3′ UT-region probe described above. The tumor blot was later stripped and rehybridized (not shown) with a [α-^{32}P]dCTP labeled GAPDH PCR-amplified probe to normalize *EHF* expression levels relative to this housekeeping gene. Express Hybridization Solution (Clontech) and short to long autoradiographs were used to detect moderate to low level gene expression.

Analysis of EHF regulatory function. The stromelysin-1 (−478 to +4) and collagenase-1 (−610 to +61) target promoters joined to the human growth hormone (GH) reporter gene in vector pFGH, and the ETS-2 expression vector, were previously described (31, 37). To construct the EHF protein expression vector, a 1.3-kb *Eco*RI–*Not*I fragment of pGEMEhf (42) containing the *Ehf* ORF from mice was ligated into the EcoRI-NotI site of pcDNA3.2, modified to provide both CMV promoter control and an N-terminal FLAG epitope (used to confirm EHF protein expression by affinity purification from transfected cell extracts). The human HepG2 hepatocarcinoma cells (ECACC No. 85011430) from the European Collection of Cell Cultures were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FCS, 2 mM glutamine, streptomycin (100 mg/ml), and penicillin (100 units/ml), then grown in 96-well microtiter plates (45). These cells were then cotransfected overnight with 100 ng/well of target promoter and *ets* factor-expressing plasmid(s) (purified twice by CsCl gradient centrifugation) using calcium phosphate methods (CellPhect Transfection kit; Amersham-Pharmacia). Cultures were washed twice and incubated 24 h in DMEM with 10% FCS plus antibiotics. To assay GH reporter gene expression, the culture medium was removed and secreted GH measured by a solid phase radioimmunoassay kit (Nichols Institute). Experiments were performed in triplicate or quadruplicate wells and repeated using two different DNA preparations.

RESULTS

Human EHF cDNA sequence. Figure 1A shows the cDNA sequence of *EHF*, the human orthologue of mouse *Ehf* (42). The mouse *Ehf* and human *EHF* ORFs exhibit 88% nucleotide and 93% aa identity. Two of eight examined human *EHF* cDNA clones possess an in-frame deletion in the ORF signifying a minor 23 aa deletion splice variant, with the deleted exon lying outside of known regulatory domains. Figure 1B shows the banding pattern of *Eco*RI-digested human and mouse genomic DNAs probed with the 3′ UT region of



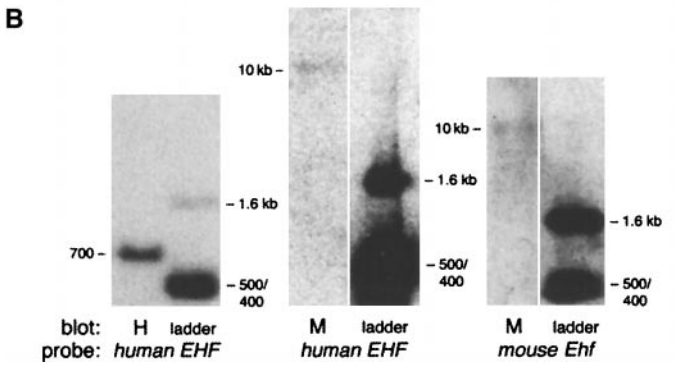


FIG. 1. Identification of single-copy human *EHF* cDNA. (A) Human *EHF* cDNA sequence. The 2590 bp sequence contains the complete EHF protein coding region (GenBank Accession No. AF170583). The putative aa sequence is shown below the nt sequence within the ORF. The nt (right) and aa (left) are numbered as indicated in the figure. Regions homologous to ets family domains are italicized and include the pointed domain (nt 240–359) and the ets domain (nt 708–965). Potential nucleotide polymorphisms in independent cDNA clones are indicated in bold: 42 (T/C); 54 (A/C); 61 (C/T); 164 (G/A); 262 (A/G) which leads to an aa change (Gln/Arg); 356 (T/C); 898 (C/T) which leads to an aa change (Ala/Val); 1124 (C/A); 1130 (A/G); 1221-1330 (deletion); and 1509 (G/C). Two of the eight clones sequenced had a splice variant where the underlined section (aa 159–181) has been deleted. The poly A addition site (boldface italics) is also shown. During final preparation of the manuscript an *EHF* search to the GenBank database identified a few additional homologous ESTs (Accession Nos. AI573169, AI554809, AI733786, AI732472), and just prior to submission, searches revealed an unpublished cDNA homologous to *EHF* (Accession Nos. AF124438, AF124439). (B) Banding pattern of Southern-blotted human and mouse *Eco*RI-cut genomic DNA hybridized with mouse and human EHF probes. Both probes hybridize to single-copy genomic bands of 10 kb (mouse) and 700 bp (human) in their respective genomes and hybridize to the same 10-kb genomic band in mouse DNA, indicating this human *EHF* clone is orthologous to mouse *Ehf*. The human *EHF* probe used for hybridization was PCR amplified using the 5′ and 3′ primers shown (underlined boldface) in A, while the mouse *Ehf* probe location encompassed adjacent ORF sequence (not shown). Band sizes are given in bp or kb, calculated from kb ladder marker DNA. Abbreviations: H, human genomic DNA; M, mouse genomic DNA.

human *EHF*, as well as mouse genomic DNA probed with an adjacent region of mouse *Ehf* as a positive control. The human *EHF* and mouse *Ehf* probes each hybridized not only to a single-copy band in genomic DNA from their respective species, but to the same single-copy band in mouse genomic DNA. Similar results were obtained with BamHI or HindIII digested genomic DNA (not shown). This confirmed that *EHF* is a single copy gene and is the actual human orthologue of mouse *Ehf*.

Human EHF maps to 11p12. To determine *EHF*'s chromosomal location we first screened human/hamster and human/mouse somatic cell hybrid templates by PCR amplification of an *EHF* 3′ UT region 309-bp fragment, which identified that *EHF* resides within chromosome 11 (data not shown). To determine *EHF*'s exact location, high-resolution FISH analysis was used. FISH detection efficiency was 58% for the 1.6-kb *EHF* 3′ UT region probe (among 100 checked mitotic figures, 58 showed hybridization signals on one pair of chromosomes). Superimposed DAPI banding photos assigned the FISH signals to the short arm of chromosome 11 (Fig. 2A), with 10/10 signals on high-resolution chromosome spreads localized within region p12 (Fig. 2B), a hotspot for genetically unassigned developmental abnormalities and a region deleted in prostate, breast and lung cancer (47, 48) (see Discussion).

Human EHF is differentially expressed in normal tissues. *EHF*'s localization in 11p12 indicates it may play a role in development and carcinogenesis. We thus determined which human tissues normally express *EHF* to shed light on its possible biological and pathological roles. An mRNA dot blot analysis showed that *EHF* is differentially expressed in normal organs, sim-

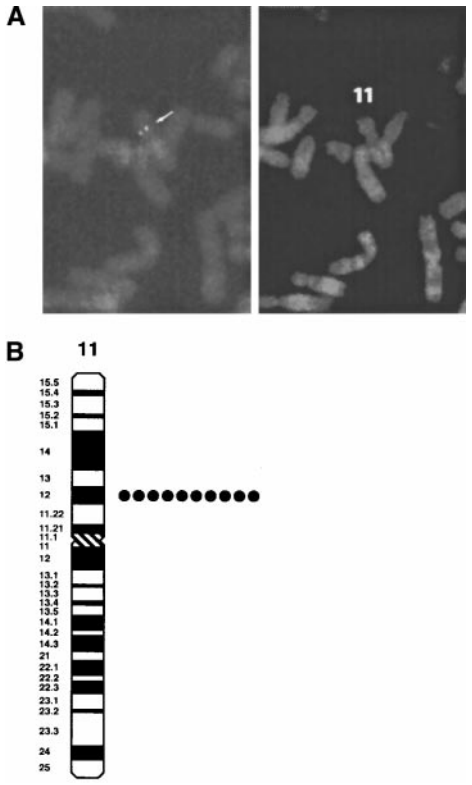
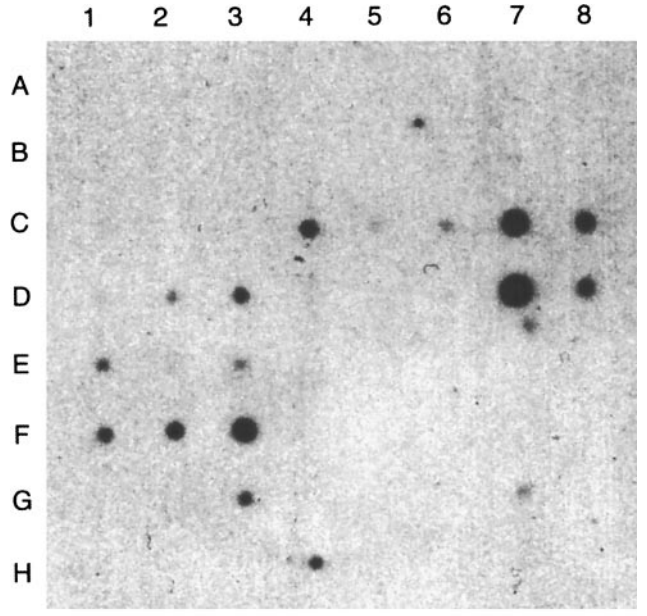


FIG. 2. Localization of human *EHF* to chromosome 11p12. (A) Left, FISH hybridization of an example mitotic chromosome spread with the 1.6 kb human *EHF* cDNA probe, showing a double FISH signal on one chromosome pair (arrow); Right, DAPI counterstaining of same mitotic figures, identifying the FISH signal's location within the short arm of chromosome 11. (B) Chromosome 11 map showing 11p12 localization of *EHF*. Dots signify DAPI-banding assignment of the location of paired FISH signals from 10 separate high-resolution mitotic chromosome spreads, indicating 10 of 10 chromosomal *EHF* FISH signals localized to 11p12.

ilar to its pattern in mice (42), and is most abundantly expressed in secretory organs such as the salivary gland, prostate and breast, and also in numerous organs or cell types containing secretory cells (Fig. 3). *EHF* was not expressed in placenta, which is rich in vascular endothelium, nor brain, suggesting its expression is not within organ vasculature. Normal prostate, lung and breast tissue, the three organs known to develop 11p12-deletion associated carcinomas, more strongly express *EHF* than most other tissues (Fig. 3). *EHF* is also differentially expressed in fetal organs, suggesting it could play a role in the development or function of some tissues prenatally.

Human EHF is differentially expressed in tumors. *EHF*'s potential role in carcinogenesis led us to examine if *EHF*, like oncogenes and tumor suppressor genes, is expressed in solid tumors and differentially expressed between tumor stages. *EHF* mRNA was differentially expressed in many different types and stages of carcinomas and other tumors both absolutely (Fig. 4) and when normalized to GAPDH expression (Fig. 5). Of the 45 tumors expressing *EHF* at detectable levels, 9 staged tumor comparisons were possible. Of these, 5/9 later-stage tumors exhibited less *EHF* expression than earlier-stage counterparts, while 4/9 exhibited more *EHF* expression. Stage-specific comparisons could not be made with the remaining 28 *EHF*-positive tumors because their stage was either unknown or not paired. Of these 28 tumors, 11 expressed less *EHF*, 11 expressed more *EHF*, and 6 had unchanged *EHF* expression when compared to normal tissue (Fig. 5).

EHF factor regulates collagenase-1 and stromelysin-1. To study the transcription-regulating activity of EHF, we examined its effect on the human stromelysin-1 and collagenase-1 promoters, which con-



Salivary Gland (D7)	++++++++++++
Prostate (C7)	++++++++++
Trachea (F3)	++++++++++
Lung (F2), Colon (C4), Stomach (C8)	+++++++++
Mammary Gland (D8)	++++++++
Pancreas (D3), Appendix (F1), Fetal Kidney (G3)	+++++++
Kidney (E1)	++++++
Ovary (D2)	+++++
Small Intestine (E3), Uterus (C6), Fetal Lung (G7)	++++
Bladder (C5), Testis (D1)	+++
Liver (E2)	++
Pituitary Gland (D4), Adrenal Gland (D5), Thyroid Gland (D6)	+
Brain Regions (A1-B6), Spinal Cord (B7), Spleen (E4), Leukocytes (E6), Bone Marrow (E8), Thymus (E5), Lymph Node (E7), Placenta (F4), Aorta (C2), Heart (C1), Muscle (C3), Fetal Brain (G1), Fetal Spleen (G5), Fetal Liver (G4), Fetal Thymus (G6), Fetal Heart (G2)	-

FIG. 3. Human *EHF* expression in normal tissues using mRNA dot blot analysis. Top, autoradiograph of an mRNA dot blot, containing polyA$^+$ RNA from multiple adult and fetal tissues in quantities normalized for equivalent expression of multiple housekeeping genes (see Materials and Methods), hybridized with a human *EHF* 3′ UT probe. Bottom, table of the relative level of *EHF* expression in each tissue. The letter/number coordinate assigned each tissue signifies the row (A–H) and column (1–8) number of the tissue's location on the dot blot. The number of "+" signs is proportionate to the level of *EHF* expression, while "−" signs signify no detected *EHF* expression.

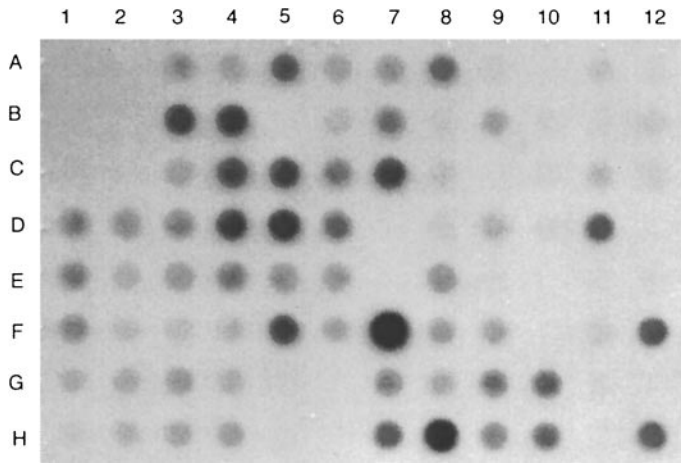


FIG. 4. Human *EHF* expression in tumor tissues using RNA dot blot analysis. Autoradiograph of an RNA dot blot, containing 5 μg of total RNA from multiple normal vs staged or unstaged tumor tissues, hybridized with a human *EHF* 3′ UT probe. Row (A–H) and column (1–12) numbers signify the letter/number coordinate assigned each tissue for detailed comparison in Fig. 5. Odd-numbered columns, tumor tissues; next highest even-numbered columns, paired normal tissues.

tain several *ets*-binding site motifs, by transient gene transfection. These promoters linked to the human GH reporter gene, pFGH (31), were cotransfected into HepG2 cells with equal amounts of plasmids expressing EHF and/or ETS-2. ETS-2 activates both promoters, as reported previously (31), in contrast to another *ets* factor, ERG, which activates collagenase-1 but strongly represses stromelysin-1 promoter activity (31, 39). Figure 6 shows that EHF activates neither promoter, although it can activate others (see Discussion). Instead, when cotransfected with ETS-2, EHF represses the ETS-2-induced activity of both promoters, indicating that EHF, like ERG, can be both a repressor and transactivator. The degree of repression by EHF on ETS-2-induced transcription also significantly differs between the two promoters, with collagenase-1 promoter expression repressed to below basal activity, but stromelysin-1 promoter expression repressed only 44%. This suggests that the extent (and possibly the mechanism) of repression by EHF depends on the promoter structure and composition of interacting transcription complexes.

DISCUSSION

EHF's localization within chromosome 11p12, a major hotspot for genetically unassigned developmental abnormalities and cancer, points to possible roles for EHF. Inherited deletions within 11p11.2-13 are associated with a spectrum of neonatal growth and mental retardation abnormalities called "DEFECT 11" syndrome (46), and in particular, deletion or LOH of 11p12 occurs frequently in carcinomas of the breast and lung (47) and in 40% of prostate carcinomas (48), as well as in acute myeloid leukemia (AML) (47), suggesting that 11p12 contains at least one tumor suppressor gene. This hypothesis was supported by microcell fusion experiments which introduced region 11p11.2-p12 into rat liver epithelial tumor cell lines, leading to suppression of tumorigenicity and transformed phenotype in one of the lines (49).

Because *EHF* resides in 11p12, it could be the 11p12 prostate, breast and lung tumor suppressor gene. *EHF* also fulfills a second major requirement for being the 11p12 tumor suppressor gene—expression in the organs that form tumors upon loss of 11p12. The only other known 11p12 gene that could conceivably act as a tumor suppressor, the transcriptional regulatory gene *Lim-1*, is detectably expressed neither in prostate nor most other adult tissues (50). In contrast, *EHF* is highly expressed in prostate, breast and lung, indeed more strongly than in almost all other organs. Hence both *EHF*'s location and normal tissue expression profile make it a candidate for the 11p12 tumor suppressor gene. Finally, *EHF* fulfills two additional characteristics of potential tumor suppressor genes—it is variably expressed in carcinomas and it can repress cancer-causing genes, in this case certain MMP genes. These issues are discussed further below.

EHF's expression in secretory organs and their solid tumors indicates it may play a role in solid carcinogenesis, unlike most other *ets* genes which are hematopoietically expressed and leukemogenic. *EHF*'s reduced expression in a majority of late-stage versus early-stage carcinomas supports the hypothesis that it could act as a tumor suppressor gene. However, of the 1 prostate, 2 breast and 5 lung tumors examined, few comparisons between stages were possible. About half of these tumors, as well as of many other tumor types of undetermined stage, likewise exhibited reduced *EHF* expression compared to normal tissue, but the other half exhibited greater *EHF* expression. Given that tumor suppressor genes can, like oncogenes, be induced in tumors before their deletion in later stages, and can exhibit cell-specific changes in their cancer-causing potential (31, 32, 51–53), these tumor data are consistent with *EHF* being either a potential tumor suppressor gene, an oncogene, or even both in separate tumors. Future studies will resolve this issue by determining EHF's efficacy in enhancing or suppressing cellular proliferation, transformation and aggressiveness *in vitro*, as well as EHF's expression changes in multiple prostate, breast and lung carcinomas.

EHF may exhibit cell- and stage-specific differences in oncogenic and tumor-suppressing potential. This is suggested not only by the variable expression of *EHF* in different solid tumors of various stages, but also by EHF's ability to both strongly repress and strongly activate different MMP genes. EHF specifically and

Tumor Blot Locale	Tissue Diagnosis	N	Tumor			
			NS	E	M	L
	Brain					
A1	Astrocytoma, Grade I		-			
B1	Neurilemmoma		-			
C1	Malig. Meningioma		-			
A-C2	Normal	-				
	Lung					
E1, D1	Squamous cell carcinoma				++	+++
G1, F1	Adenocarcinoma				+/-	+++
H1	Bronchi-Alveolar Carcinoma		+/-			
D-H2	Normal	+				
	Throat					
A3	Squamous cell carcinoma		++			
A4	Normal	+				
	Esophagus					
C3, B3	Squamous cell carcinoma				+	++
D3	Adenocarcinoma				+	
B-D4	Normal	++				
	Stomach					
E3	Single ring cell carcinoma		+/-			
H-G-F3	Adenocarcinoma			++++	+++	++
E-H4	Normal	+				
	Duodenum					
A5	Adenocarcinoma		+++			
A6	Normal	+				
	Small Intestine					
B5	Malig. mesothelioma		+			
B6	Normal	+				
	Colon					
D5, C5	Adenocarcinoma			+++		++
C-D6	Normal	+				
	Rectum					
F5, E5	Adenocarcinoma				+++	+
E-F6	Normal	+				
	Liver					
H5, G5	Hepatocellular carcinoma				+/-	+
G-H6	Normal	+/-				
	Gallbladder					
A7	Squamous cell carcinoma			+		
A8	Normal	+				
	Pancreas					
B7	Adenocarcinoma		++			
B8	Normal	+				
	Parotid					
C7	Pleomorphic adenoma		++			
C8	Normal	+				

Tumor Blot Locale	Tissue Diagnosis	N	Tumor			
			NS	E	M	L
	Kidney					
D7	Granular cell carcinoma		+/-			
E7	Clear cell carcinoma		+/-			
D-E8	Normal	+				
	Bladder					
F7, G7	Transitional cell carcinoma				+++	++
F-G8	Normal	+				
	Prostate					
H7	Hyperplasia		+			
H8	Normal	+++				
	Testis					
A9	Seminoma		+			
A10	Normal	+/-				
	Ovary					
B9	Muc. cystadenocarcinoma		+++			
C9	Thecoma		+/-			
D9	Teratoma		++			
B-D10	Normal	+				
	Uterus					
E9	Leiomyoma		+/-			
F9	Adenocarcinoma		++			
E-F10	Normal	+/-				
	Breast					
G9	Invasive ductal carcinoma		+++			
H9	Fibradenoma		+			
G-H10	Normal	++				
	Thyroid					
A11	Nodular goiter of thyroid		+			
B11	Follicular adenoma		++			
C11	Papillary adenocarcinoma		+			
A-C12	Normal	+				
	Adrenal					
D11	Neuroendocrinic carcinoma		+++			
D12	Normal	+				
	Thymus					
E11	Thymoma		+/-			
E12	Normal	+/-				
	Lymph Nodes					
F11	Lymphoma of Tonsil		+/-			
G11	Non-Hodgkin's Lymphoma		+/-			
F12	Normal	++				
G12	Normal	+/-				
	Soft Tissue					
H11	Malig. fibrous histocytoma		+/-			
H12	Normal	++				

FIG. 5. Relative human *EHF* expression levels in tumors. Shown is the relative level of human *EHF* expression, after normalizing to GAPDH levels, between staged tumors or between unstaged tumors compared to paired normal tissues, based on Fig. 4. The letter/number coordinates assigned each tissue signify the row (A–H) and column (1–12) numbers of the tissues' locations on the dot blot. The number of "+" signs is proportionate to the level of human *EHF* expression. The "+/−" signs signify reduced expression relative to "+" signs, while "−" signs signify absent *EHF* expression. Abbreviations: N, normal tissue; NS, non-staged tumor tissue; E, early-stage tumor tissue; M, middle-stage tumor tissue; L, late-stage tumor tissue.

differentially represses the ETS-2-induced activity of stromelysin-1 and collagenase-1 promoters, even though under the same conditions EHF is a strong activator of the collagenase-3 promoter (Duggan and Butticè, in preparation). We have previously shown that ERG complexed with ETS-2 binds the two *ets*-binding sites in the stromelysin-1 promoter and represses its transcription (39), while ERG complexed to Fos/Jun binds to the composite EBS/AP-1 site in the collagenase-1 promoter and activates this gene (31). As with ERG, EHF may also differentially regulate MMP and other cancer genes in different tissues and tumor cells, by varying the extent and direction of its influence on other transcription factors.

Ehf was first discovered in G_s-induced pituitary somatotroph tumors in mice (42), and its expression in this slow-growing, benign tumor tissue hinted at a possible role for EHF in inducing or suppressing solid tumors of secretory cells. EHF, along with its closest yet still distant relatives, ESX and ELF5, comprise a newly discovered but ancient *ets* subfamily expressed in solid tissues and thought to cause clinically prevalent carcinomas, in contrast to earlier characterized *ets* factors which are hematopoietically-expressed and cause leukemias (26, 42). ESX is expressed in mammary secretory epithelium, up-regulated in early-stage breast cancer, and induces *HER2/neu* oncogene transcription (21), suggesting ESX induces secretory epithelium-derived carcinomas. ELF5 is also epithelium-expressed, but thought to transactivate tumor suppressors like prostate maspin to suppress prostate carcinoma growth (26). *ELF5* and *EHF* both map to distinct tumor suppressor sites on chromosome 11, and are most strongly expressed in the normal organs that form solid tumors upon loss of these sites (prostate, breast and lung for *EHF;* kidney and prostate for *ELF5*). *ELF5* expression is also lost in prostate tumors compared to surrounding normal tissue, while *EHF* expression was greatly diminished in the single prostate tumor sample examined in our multi-tumor survey. EHF can also inhibit ETS-2, which is required to maintain the transformed state of prostate carcinoma cells (54). ESX and ELF5 have exhibited transactivator ability, while EHF can repress or transactivate different cancer-causing genes, which may explain its differential but variable expression in many carcinomas.

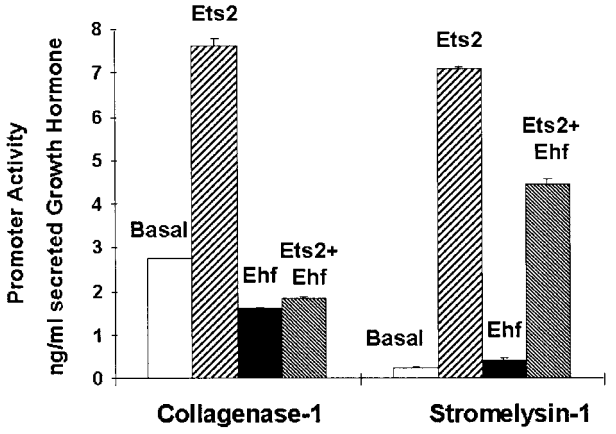


FIG. 6. EHF regulation of collagenase-1 and stromelysin-1. Human collagenase-1 (−610 to + 61) and stromelysin-1 (−478 to +4) gene promoters were linked to the growth hormone reporter gene and studied in transiently transfected HepG2 cells. Equal amounts of empty plasmid (as negative control) and plasmids expressing ETS-2 or EHF were cotransfected with the MMP promoter constructs. After transfection the cells were incubated for 24 h and the quantity of secreted growth hormone was measured by radioimmunoassay. As a control, the promoterless reporter gene (pFGH) was studied under the same conditions. The graph shows data from a representative experiment. The values are the average of triplicate transfections expressed as ng/ml of secreted growth hormone. Standard error bars indicate the variation between triplicate transfections.

Overall, our results are consistent with the possibility that EHF could facilitate carcinogenesis in multiple organs, including those exhibiting 11p12 deletion-associated carcinomas, but could do so either as a tumor suppressor or oncoprotein, or both, depending on its differential transcription factor interactions within different tissues. Current data on ESX, ELF5 and EHF support the idea that each member of this new *ets* subfamily, although distantly related and possibly regulating various *ets*-responsive cancer genes differently, probably plays a significant role in major human carcinomas.

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