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HNF1α upregulates the human AE2 anion exchanger gene (*SLC4A2*) from an alternate promoter

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Abstract

The human AE2 gene (*SLC4A2*) is transcribed in a widespread fashion from the upstream promoter, the resultant full-length transcript AE2a being encountered in most tissues. Moreover, alternate promoter sequences within intron 2 may drive tissue-restricted expression of variants $AE2b_1$ and $AE2b_2$, mainly in liver and kidney. $AE2b_2$ proximal promoter sequences are highly active in transfected liver-derived HepG2 cells and contain an HNF1 motif. Mutation-disruption of this motif dramatically decreased alternate promoter activity in HepG2 cells but not in prostate-derived PC-3 cells. Electromobility shift and supershift assays indicated that HNF1α from HepG2 nuclear extracts binds the HNF1 sequence. Transactivation studies in PC-3 cells showed enhanced activity of the wild-type construct upon cotransfection with an HNF1α expression plasmid, while activity of the HNF1-mutated construct remained unaffected. Since liver AE2 is putatively involved in the biliary secretion of bicarbonate, HNF1α may have a role in increasing bicarbonate secretion in response to certain stimuli.

Keywords: Human AE2; Alternate promoter; Chloride/bicarbonate exchange; Hepatocyte nuclear factor 1α; Liver-derived HepG2 cells; Prostate-derived PC-3 cells; Tissue-specific expression

Na^+-independent anion exchangers (AE) are membrane proteins that mediate electroneutral and reversible exchange of Cl^- and HCO_3^- across the plasma membrane [1,2]. AE polypeptides usually load Cl^- into the cell and extrude HCO_3^-, promoting intracellular acidification. In cooperation with other ion carriers, they are involved in the regulation of intracellular pH, cell volume homeostasis, and transepithelial acid/base transport. To date, four members of the AE family (AE1, AE2, AE3, and AE4) have been characterized in several species [2–5]. The corresponding genes show similarities in size, sequence, and exon/intron organization, although they are located in different chromosomes. A common feature among AE genes is the usage of alternative promoters, which lead to the expression of alternative transcripts, most often in a tissue-specific manner [1]. Thus, a 5′-truncated variant of the erythroid AE1 mRNA [6–9] is expressed in the kidney, while an AE3 transcript is found in cardiac muscle as a 5′-truncated variant of the complete form, which is mainly expressed in brain [10–13]. Concerning AE2, the complete message transcribed from the upstream promoter (AE2a) has been encountered in all human tissues that have been explored (liver, stomach, thyroid, prostate, and kidney), while expression of two 5′-truncated AE2 mRNAs ($AE2b_1$ and $AE2b_2$) was only found in liver and kidney [14]. Alternative exons $1b_1$ and $1b_2$ are transcribed from $AE2b_2$/$AE2b_1$ overlapping promoter sequences within intron 2 [14], each one splicing to exon 3 in corresponding 5′-truncated variants. As a result of the 5′-diversity of human AE2 mRNAs, the first 17 amino acids of AE2a are replaced by three initial residues (MTQ) in $AE2b_1$ and by eight initial residues (MDFLLRPQ) in $AE2b_2$. While former experiments

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showed the capability of the AE2a message to be functionally expressed in human cells [15,16], recent experiments provide similar evidence for the alternative messages $AE2b_1$ and $AE2b_2$.

AE2 is the member of the AE family putatively involved in the biliary secretion of bicarbonate. Immunohistochemical studies in human liver using a monoclonal antibody against a peptide common to all three AE2 isoforms showed immunoreactivity at the lumenal surface of the hepatobiliary tree only [17]. Therefore, it seems that all three AE2 isoforms have an apical location, each being able to participate in the secretion of bicarbonate to bile. In this context, the use of alternate promoter sequences may result in differentially regulated expression of AE2 isoforms in hepatobiliary cells. Previous dual-luciferase reporter gene assays of $AE2b_2$/$AE2b_1$ overlapping promoter regions in transiently transfected HepG2 cells determined that a 310-bp region corresponding to the proximal promoter of $AE2b_2$ is highly active [14]. This region contains several potential binding motifs for liver-enriched factors, including the sequence TCTTAATGATTAACC, which is a 1-bp variant (underlined) of the 15-bp consensus sequence for hepatocyte nuclear factor-1 (HNF1) [18]. There are two liver-enriched transcription factors, HNF1α and HNF1β, that may bind the HNF1 element as homo- or heterodimers [19–21]. Both HNF1α and HNF1β are expressed mainly in liver and kidney, but also in intestine and pancreas [22], while HNF1β alone is found in lung and gonads [23,24]. Liver HNF1α has been recently reported to be a master player in regulating bile acid and cholesterol metabolism [25,26].

Here, we determined that HNF1α can: (i) bind the HNF1-motif variant of the human AE2 alternate promoter region and (ii) transactivate alternative transcription of the AE2 gene. Our findings suggest that HNF1α may have a major role in upregulating alternative transcription of the human AE2 gene in the liver, and therefore it may contribute to the biliary secretion of bicarbonate in response to certain stimuli.

Table 1
Oligonucleotides used for the analysis of mRNA expressions by real-time PCR

Oligonucleotide	Sequence (5′ to 3′)
GAPDH forward	CCAAGGTCATCCATGACAAC
GAPDH reverse	TGTCATACCAGGAAATGAGC
AE2a forward	TCCAGAGCGAGCGGGTTATG
$AE2b_1$ forward	CGCCCGCAGGATGACTCA
$AE2b_2$ forward	CCCTCCTTCTCAGGTTCACCCCTGCC
AE2 reverse (common for all isoforms)	GAGGACTGGCGGTGGTACTCAAAGTC
HNF1α forward	TGGGTCCTACGTTCACCAAC
HNF1α reverse	TCTGCACAGGTGGCATGAGC
HNF1β forward	TGCACAAAGCCTCAACACCT
HNF1β reverse	GTTGGTGAGTGTACTGATGC

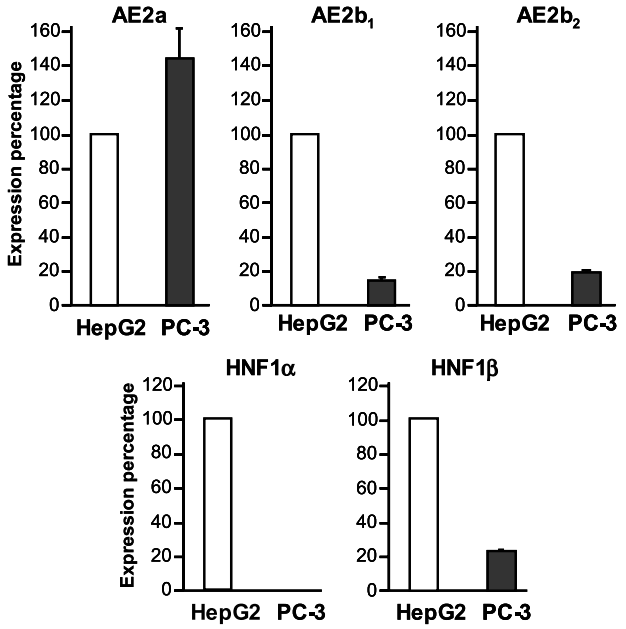

Fig. 1. Comparisons between HepG2 and PC-3 cells in the expression of mRNA isoforms for AE2 and HNF1. After reverse transcription of total RNA, expression of mRNA isoforms was measured by real-time PCR using specific primer pairs (cf. Table 1). Data were normalized with GAPDH. The levels of transcript expressions in PC-3 cells are given as percentage (means ± SEM of at least three samples) of those in HepG2, which are each noted as 100%.

Materials and methods

Human cell lines. HepG2 cells (from human hepatoblastoma) were grown in DMEM with glutamax, while PC-3 cells (from human prostate cancer) were grown in RPMI 1640 with glutamax (Gibco). Both media were supplemented with 10% fetal calf serum (Gibco), penicillin/streptomycin (BioWhittaker), and 10 mM Hepes (Sigma), pH 7.4, at 37 °C.

Isolation of total RNA and real-time polymerase chain reaction. Total RNA was isolated from each cell line with the TRI Reagent (Sigma). Reverse transcription of total RNA (2 μg) and real-time polymerase chain reaction (PCR) were performed as described [27], using specific primers (Sigma-Genosys) (cf. Table 1 and Fig. 1).

Transient transfections with promoter–luciferase constructs. PCR fragments corresponding to alternate promoter sequences were obtained as described [14], using specific oligonucleotides (Table 2). A promoter fragment with a 6-bp disrupted HNF1 motif was obtained through a PCR-based mutagenesis protocol [28] employing internal mutated oligonucleotides (Table 2). Promoter fragments were ligated to *Sma*I-linearized vector pGL3-basic (Promega), the integrity of each insert being further confirmed by sequence analysis. Transient transfections and assessments for luciferase activities with the Dual-Luciferase Reporter Assay System (Promega) were carried out as described [14], with minor modifications. Briefly, cells were seeded in 24-well culture plates (8×10^4 HepG2 cells/well or 4×10^4 PC-3 cells/well) one day before transfections. HepG2 cells were transfected by the calcium phosphate precipitation method [29], using for each well 0.2 μg of the corresponding promoter construct, 1 ng of the internal standard pRL-SV40 construct, and 1.9 μg of carrier DNA (vector pGL3 basic). PC-3 cells were transfected with 0.4 μg of the corresponding promoter

Table 2
Oligonucleotides used for the production of promoter fragments by PCR

Oligonucleotide	Orientation	Sequence (5′ to 3′)
$-471b_2$	Forward	CCTACCCTGACTTTGCATGA
$-286b_2$	Forward	TAGAGGCGCAGGTCAAGTCT
$+24b_2$	Reverse	TGAGGCCGCAGGAGGAAGTCCAT
$-143b_2$	Reverse	CAAAGGGGGCCATGTGGAGC
Int-Mut-HNF1	Forward	AGCGCCTCACGCCGTGCCACAATCAGCTCC
Int-Mut-HNF1	Reverse	GCGTGAGGCGCTAAGACTCCTTAGGAAAATC

Oligonucleotide name indicates the position of each oligonucleotide 5′-end in the promoter sequence referred to the corresponding start codon. The last two are internal oligonucleotides used in the mutagenesis procedure (underlined nucleotides indicate mutations that disrupted the HNF1 element).

construct and 2 ng of pRL-SV40 per well, using the Tfx-50 Reagent (Promega). For cotransfection assays with PC-3 cells, 0.2 μg per well of either pBJ5-HNF1α or the empty vector pBJ5 (kindly provided by Dr. Gerald Crabtree [30]) was added to the transfection reaction. Forty-two hours after transfections, dual-luciferase activities were assayed in a Lumat LB 9507 luminometer (EG&G Berthold).

Preparation of nuclear extracts and electromobility shift assays. HepG2 or PC-3 cells seeded in 150 × 20 mm culture plates (TPP) were grown until 70–80% confluence and collected with a Costar cell scraper. Cell centrifugation and following steps to recover the nuclear proteins were all performed at 4 °C. The cellular pellet was resuspended in a lysis buffer (10 mM Hepes, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT from Sigma, and 1× Complete Protease Inhibitor Cocktail Tablets from Roche). After 15-min incubation, cells were homogenized and nuclei were pelleted (12,000*g* for 1 min). The pellet was resuspended in appropriate buffer (20 mM Hepes, pH 7.9, 25% glycerol, 1.5 mM $MgCl_2$, 420 mM NaCl, 0.2 mM EDTA, 1 mM DTT, and protease inhibitor as above) and incubated for 30 min, with brief vortexing every 5 min. Nuclear membranes were pelleted (12,000*g* for 5 min) and the supernatant was dialyzed for 2 h at 4 °C against 50 ml of a dialysis buffer (20 mM Hepes, pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 1 mM DTT, and protease inhibitor). Following centrifugation at 13,000*g* for 5 min, the supernatant was stored at −80 °C. Nuclear extracts from PC-3 cells transfected with either the expression vector pBJ5-HNF1α or the empty vector pBJ5 were also prepared; 150 × 20 mm culture plates were seeded with 7.2×10^5 cells, each plate being transfected the following day with 36 μg of corresponding vector using Tfx-50 Reagent (Promega). Forty-two hours after transfection nuclear proteins were extracted as described above. Protein concentrations were measured by using the Bradford method (Bio-Rad).

For electromobility shift assays (EMSAs), the double-stranded probe 5′-CCTAAGGAGTCTTAATGATTAACCCCGTGCC-3′ (HNF1 motif underlined) was produced by annealing of partially complementary oligonucleotides HNF1 forward and HNF1 reverse (Table 3), followed by 3′-end filling with Klenow (New-England Biolabs). Unincorporated nucleotides were removed with a Nick Column (Amersham) and digoxigenin-11-ddUTP was incorporated to each 3′-end using the Dig Gel Shift Kit (Roche). Mutated probe was generated with oligonucleotides mutated-HNF1 forward and mutated-HNF1 reverse (Table 3), containing the same changes as the promoter construct with a disrupted HNF1 motif; cf. above). Each probe (0.2 pmol) was incubated in a 20-μl mixture with 1 μg of poly(dI-dC) (Amersham), 8–16 μg of nuclear extract from non-transfected cells, 12 mM Hepes, pH 7.8, 0.1 M KCl, 1 mM EDTA, 1 mM DTT, 5% glycerol, and 50 ng BSA. For competition assays, an excess of unlabeled probe (1-, 10- or 30-fold) was added. As non-specific DNA-binding competitor an unlabeled probe with an Oct2A-binding site was used. Supershift experiments included the addition of 4 μg of anti-HNF1α or anti-HNF1β antibodies (Supershift reagent sc-6548 X or sc-7411 X, respectively, Santa Cruz Biotechnology). Binding reactions were incubated for 15 min at room temperature before electrophoresis on a 4% polyacrylamide gel. After electrotransference to a positively charged nylon membrane (Roche), bands were detected non-isotopically with the Dig Gel Shift Kit and exposed to an autoradiography film for at least 30 min.

Table 3
Oligonucleotides used for the production of HNF1 probes for EMSA

Oligonucleotide	Sequence (5′ to 3′)
HNF1 forward	CCTAAGGAGTCTTAATGATTAACCCC
HNF1 reverse	GGCACGGGGTTAATCATTAAGACTC
Mutated-HNF1 forward	CCTAAGGAGTCTTAGCGCCTCACGCC
Mutated-HNF1 reverse	GGCACGGCGTGAGGCGCTAAGACTC

Underlined nucleotides indicate mutations that disrupt the HNF1 binding site.

When using nuclear extracts from PC-3 transfected cells, a lower amount was prepared and isotopic EMSA was performed to increase the sensitivity. The HNF1 probe was labeled with [^{32}P]ATP (Amersham) and T4 polynucleotide kinase (Promega). Binding buffer and conditions were the same as for non-isotopic EMSA, but only 0.02 pmol of probe and 2 μg of nuclear extract were used. After PAGE, the gel was dried and exposed to an autoradiography film for at least 42 h.

Western blot. Eight micrograms of nuclear extracts in 66 mM Tris, 100 mM DTT, 2.6% SDS, 4.8 M urea, 10% glycerol, and 10 mg/ml of bromophenol blue was run in 0.1% SDS–6% PAGE and electrotransferred to a Biotrace NT membrane (Pall). Sequential incubations were carried out with anti-HNF1α (sc-6548 X diluted 1/8000) and anti-goat IgG-HRP (a donkey antibody from Santa Cruz Biotechnology, diluted 1/100,000). Western Lightning (Perkin–Elmer) and *Hyperfilm ECL* (Amersham) were used for visualization of the bands.

Results

Comparative studies between HepG2 and PC-3 cells for the expression of AE2 and HNF1 mRNA isoforms

Real-time quantitative PCR showed that the steady-state levels of AE2a mRNA were higher (144 ± 18%) in prostate-derived PC-3 cells than in liver-derived HepG2 cells, whereas the levels of both alternative messages $AE2b_1$ and $AE2b_2$ were significantly lower (14 ± 2% and 18 ± 2%, respectively) in prostate cells (Fig. 1). The low expression of alternative transcripts in PC-3 cells was associated with low levels of HNF1 transcripts in these cells compared to HepG2 cells. In fact, expression of HNF1α mRNA was undetectable in prostate cells and the levels of HNF1β mRNA were significantly lower (23 ± 1%) in these cells than in HepG2 (Fig. 1).

Involvement of the HNF1 motif in the alternate transcriptional activity of AE2

We assessed the functionality of the 1-bp variant of the HNF1 consensus sequence spanning positions $-127b_2$ to $-113b_2$ in the minimal $AE2b_2$ promoter [14]. Thus, we carried out transfections of HepG2 cells with promoter constructs either lacking the region with the HNF1 sequence (see the 3′-deleted construct $-286b_2/-143b_2$ in Fig. 2) or containing a disrupted HNF1 motif with six mutations of the consensus sequence ($-286b_2/+24b_{2Mut}$). Dual-luciferase assays with transfected HepG2 cells showed that transcriptional activities were markedly attenuated versus cells transfected with the wild-type construct $-286b_2/+24b_2$ ($28 \pm 1\%$ and $32 \pm 3\%$, respectively; both $p < 0.001$, cf. Fig. 2). In PC-3 cells, luciferase activities were much lower (approximately 50-fold) than in HepG2 cells. In PC-3 cells, however, HNF1-motif disruption in $-286b_2/+24b_{2Mut}$ had no effect ($101 \pm 9\%$ versus wild-type construct $-286b_2/+24b_2$; data not shown), indicating that the HNF1 motif is not functional in these cells.

Binding of HNF1α to the HNF1 motif

EMSA with HepG2 nuclear extracts and a DIG-labeled 31-bp probe containing the HNF1 sequence (and flanking bases in the promoter) resulted in two shifted bands (Fig. 3A). Only the upper shifted band was specific, since it could be competed with unlabeled specific probe, but not with an unlabeled probe having a disrupted HNF1 motif or with the non-specific oligonucleotide Oct2A (Fig. 3A). Furthermore, the upper shifted band was not obtained when performing EMSAs with the labeled probe containing the disrupted version of the HNF1 site (Fig. 3B). These data show that our 1-bp variant of the HNF1 element is able to bind nuclear proteins from HepG2 extracts. Interestingly, EMSA using a labeled probe with the C at the second nucleotide (the 1-bp variant of the HNF1 consensus sequence) mutated to G (the consensus nucleotide) showed a similar binding than the wild-type HNF1 probe (Fig. 3B).

When supershift assays with antibodies to HNF1α and HNF1β were carried, a supershift of the specific shifted band was only observed with the HNF1α antibody but not with the HNF1β antibody (Fig. 3C). Therefore, in HepG2 cells the HNF1 element appears to be bound by HNF1α/HNF1α homodimers.

No specific shifted band was detected in EMSA with PC-3 nuclear extracts instead of HepG2 extracts (Fig. 4A). This was expected because HNF1α is not expressed in PC-3 cells, as indicated by real-time PCR experiments (Fig. 1). Western blot performed with nuclear extracts confirmed that HNF1α is present in HepG2 but not in PC-3 cells (Fig. 4B). On the other hand, immunoblot analysis of PC-3 cells transfected with an HNF1α-expressing vector showed expression of

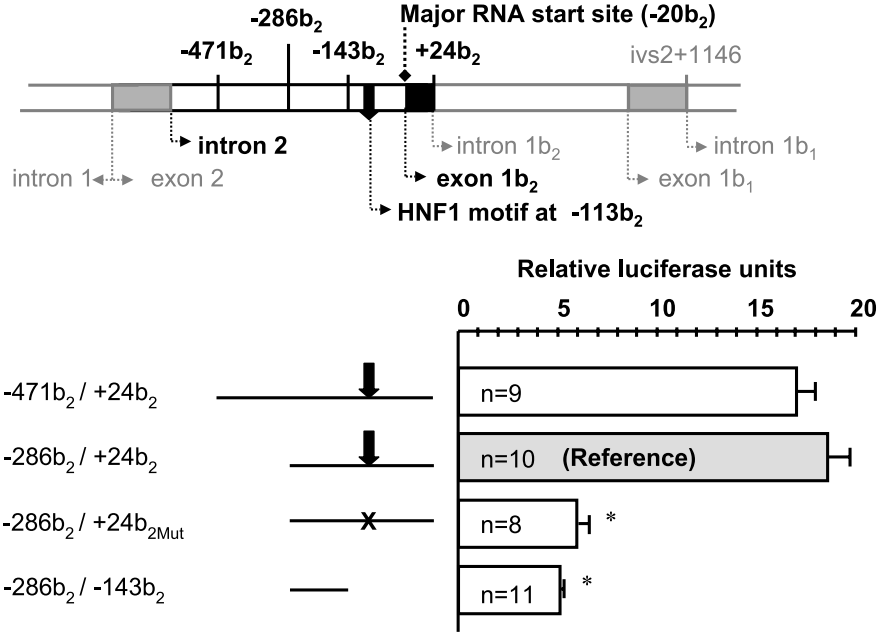

Fig. 2. Luciferase activities of constructs with sequences of the human alternate overlapping promoters $AE2b_2/AE2b_1$. The upper diagram shows the promoter regions from the distal part of intron 1 to the end of exon $1b_1$. Dual-luciferase assays of HepG2 cells transiently transfected with the corresponding constructs were carried out in triplicate and repeated several times (indicated by the numbers inside the bars). For each experiment, relative luciferase units were normalized with values obtained for the reference construct $-286b_2/+24b_2$; results are given as means ± SEM. Statistical significance (*) of differences to the reference construct was determined by Student's *t* test with the Bonferroni correction ($\alpha = 0.01$). Black arrows indicate the location of the HNF1 motif; ✕ indicates the mutagenesis-disrupted HNF1 site.

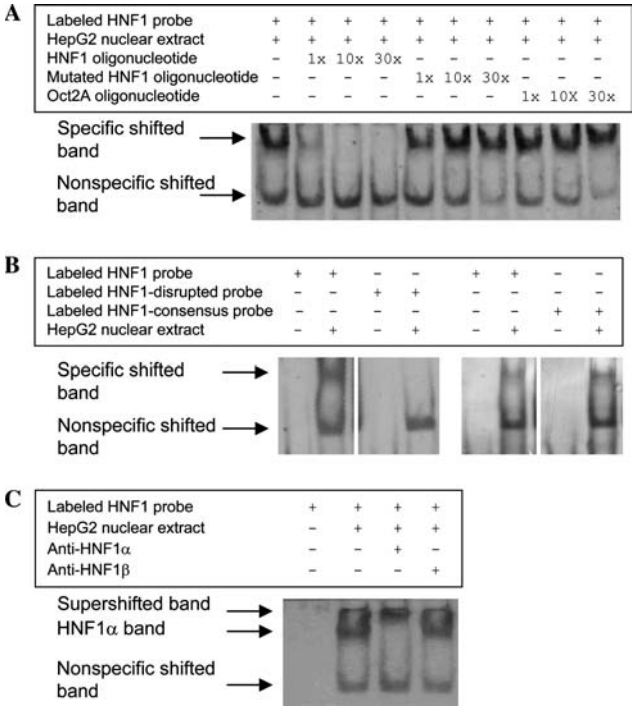

Fig. 3. HNF1α transcription factor from HepG2 nuclear extract is able to bind to the HNF1 element. (A) EMSA using nuclear extracts from HepG2 cells and competition assays with several oligonucleotides, i.e., unlabeled HNF1 oligonucleotide, mutated oligonucleotide with a disrupted HNF1 site, and oligonucleotide with an Oct2A element. Each competitor was assayed at 1, 10 or 30 molar excess with respect to the labeled probe. (B) EMSA with mutated labeled probes: using a probe with the HNF1 element disrupted by a 6-bp mutation resulted in no specific shifted band; experiments using an HNF1 probe with the C at the second nucleotide mutated to G (the consensus nucleotide) showed a similar binding than the wild-type HNF1 probe. (C) Supershift experiments carried out with antibodies to HNF1α and HNF1β indicate that HNF1α is binding to the HNF1 element. All these EMSAs were performed non-isotopically with digoxigenin-labeled probes.

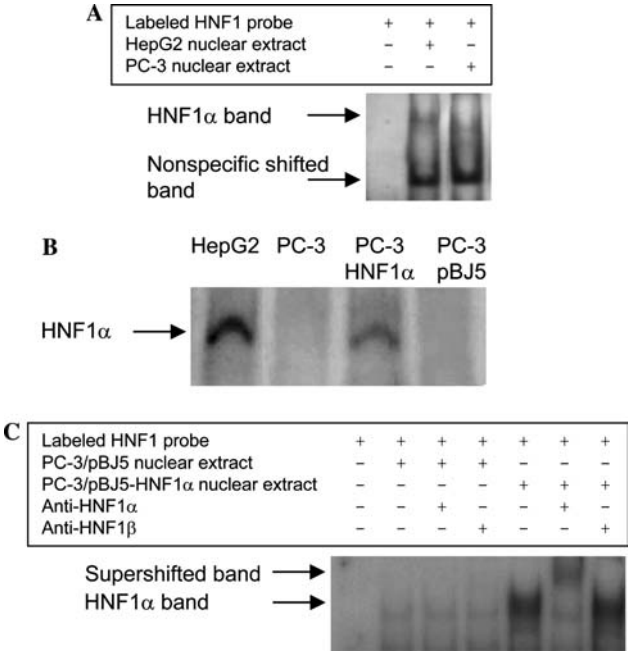

Fig. 4. Transfection with an HNF1α vector and expression of HNF1α in PC3 cells. (A) In contrast to the experiments with nuclear extract from HepG2 cells, EMSA with nuclear extract from PC-3 cells does not show the upper shifted band due to HNF1α; this EMSA was performed non-isotopically with a digoxigenin-labeled probe. (B) Western blot for the immunodetection of HNF1α in nuclear extracts from HepG2 and PC-3 cell lines: while HNF1α is present in HepG2 cells, this transcription factor is not detected in PC-3 cells prior to their transfection; transfection with pBJ5-HNF1α induces the appearance of HNF1α in the nucleus of PC-3 cells. (C) EMSA using nuclear extracts prepared from PC-3 cells transfected with pBJ5-HNF1α; a major shifted band equivalent to that observed when using nuclear extracts from HepG2 cells was supershifted with the anti-HNF1α antibody. This EMSA was performed using a ^{32}P-labeled probe.

HNF1α in these cells (Fig. 4B). EMSA and supershift studies using nuclear extracts from PC-3 cells transfected with the HNF1α vector demonstrated the occurrence of HNF1α binding activity in these transfected cells (Fig. 4C).

Transactivation studies in PC-3 cells

To demonstrate that HNF1α may transactivate the human alternate AE2 promoter, wild-type construct $-286b_2/+24b_2$ and the HNF1-mutated counterpart $-286b_2/+24b_{2Mut}$ (Fig. 2) were each cotransfected with either the pBJ5-HNF1α vector or the empty vector pBJ5 into PC-3 cells (Fig. 5), which do not express HNF1α (cf. Figs. 1 and 4B). Luciferase activity of cells transfected with the wild-type construct was markedly increased when cotransfected with the expression vector for HNF1α ($231 \pm 18\%$, $p < 0.001$; see Fig. 5), whereas no increase was observed in cells cotransfected with the empty vector pBJ5 ($96 \pm 9\%$, not significant). On the other hand, the

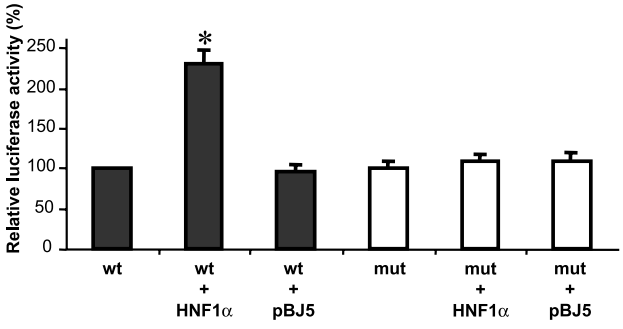

Fig. 5. Cotransfection with an HNF1α-expressing vector transactivates the wild-type $AE2b_2$ proximal promoter in PC3 cells. Dual-luciferase assays using PC-3 cells cotransfected with the expression vector pBJ5-HNF1α or the empty vector pBJ5 and either the wild-type (wt) HNF1 containing construct $-286b_2/+24b_2$ or its mutated (mut) version in construct $-286b_2/+24b_{2Mut}$, were carried out in triplicate and repeated at least nine times. Values are all given as percentage of the reference construct $-286b_2/+24b_2$. Mean values are represented and the error bars are the standard error of the mean. Statistical significance (*) of differences was determined by Student's t test with the Bonferroni correction ($\alpha = 0.01$).

activity of mutated construct $-286b_2/+24b_{2Mut}$ was not affected by any cotransfection, remaining similar to that of the wild-type construct transfected alone into PC-3 cells ($109 \pm 10\%$ and $108 \pm 10\%$ for pBJ5 and pBJ5-HNF1α cotransfections, respectively, both not significant; Fig. 5). Thus, cotransfection experiments in PC-3 cells demonstrate that HNF1α is able to transactivate the $AE2b_2$ proximal promoter.

Discussion

The human AE2 anion exchanger gene is widely expressed from its upstream promoter AE2a. In the liver as well as in the kidney, this gene may also be alternatively expressed from overlapping promoter sequences $AE2b_2/AE2b_1$ within intron 2 [14]. In the present study, we have determined that liver-enriched nuclear factor HNF1α plays an important role for transactivating the alternate promoter $AE2b_2$ in liver-derived HepG2 cells, and that HNF1α exerts this function through its binding to the HNF1 motif in the alternate promoter $AE2b_2$ [14]. Moreover, our data from real-time PCR and luciferase studies show that this is not the case in prostate-derived cell line PC-3, and indicate that HNF1α-mediated alternative transcription of the AE2 gene in humans is rather cell specific, similar to what has been recently reported in rabbit gastric mucosa for the different cell types [31].

The HNF1 motif located at position $-113b_2$ in the overlapping alternate promoter sequences $AE2b_2/AE2b_1$ follows the most stringent criteria reported for bona fide HNF1 sites [18]. Thus, it locates in the minimal $AE2b_2$ promoter region and is particularly surrounded by other transcription factor motifs [14]. However, our HNF1 motif contains a C at the second nucleotide position in the consensus sequence that, to our knowledge, has never been found among hundreds of them [18]. EMSA experiments showed that nuclear extract from HepG2 cells binds similarly either HNF1-probes with C or G at the second nucleotide position (Fig. 3B).

The HNF1 element is known to be able to interact with homo- or heterodimers of HNF1α and HNF1β [19–21]. They both are factors included in the wide HNF network [32,33] that may regulate tissue-restricted expression of some genes [34–40]. Hypothetically, either HNF1α and/or HNF1β could be responsible for the transcriptional activation of the AE2 alternate promoter in liver-derived HepG2 cells. However, supershift assays indicate that the HNF1 element is mainly bound by HNF1α/HNF1α homodimers in these liver cells (Fig. 3C). In prostate cells, the lack of HNF1α binding activity (Fig. 4A) correlates well with the absence of HNF1α expression in these cells (Figs. 1 and 4B). PC-3 cells transfected with an HNF1α vector to express HNF1α (Fig. 4B) showed HNF1α binding activity as determined by EMSA and supershift studies (Fig. 4C). Cotransfection experiments in these prostate cells definitely demonstrated that HNF1α can transactivate the $AE2b_2$ proximal promoter (Fig. 5). These experiments also suggest that cell-specific alternative AE2 expression is largely influenced by the presence or absence of HNF1α, although other transcription factors are most probably involved as well. Thus, cells lacking HNF1α, such as PC-3, show low but detectable expression of alternative transcripts (Fig. 1), and disruption of the HNF1 motif does not fully eliminate transcriptional activity of the $AE2b_2$ proximal promoter in any of the two cell lines tested.

Former studies have demonstrated that, in basal conditions, expression of alternative messages $AE2b_1$ and $AE2b_2$ accounts each for about 10% of the full-length AE2a expression [14], which is driven from upstream promoter sequences that resemble house-keeping gene promoters [41]. The physiological significance of an alternative expression of the AE2 gene in the liver regulated by HNF1α might be related to the possibility for liver cells to increase the expression of the AE2 gene when needed for bicarbonate secretion. Biliary secretion of bicarbonate occurs through a Na^+-independent anion exchange along the apical membrane of hepatobiliary cells. All three AE2 isoforms may be involved in this exchange, as recent studies in HEK293 cells transfected with AE2-isoform-expressing vectors indicate that each isoform is able to display AE activity at a similar degree (V. Aranda, S.M., and J.F.M., unpublished).

The role of HNF1α in directing the expression of genes involved in bile acid transport has been recently highlighted. The livers of Hnf1α (*Tcf1*) knockout mice show decreased expression of several basolateral membrane bile acid transporter genes such as *Slc10a1* (*Ntcp* or sodium taurocholate cotransporting polypeptide gene), *Slc21a3* (*Oatp1* or organic anion transporting polypeptide-1 gene), and *Slc21a5* (*Oatp2*), leading to impaired portal bile acid uptake [25]. Moreover, the promoter regions of *Oatp4* and its human ortholog *OATP-C* have been shown to be responsive to HNF1α [42]. These and other studies [26,43,44] provide support for an important role of HNF1α as a key regulator of liver-specific organic anion transporter genes and hence for the production of the bile acid-dependent bile flow. Likewise, our present report provides evidence that HNF1α is able to regulate a gene involved in biliary secretion of bicarbonate, and thus in the generation of the bile acid-independent bile flow.

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