Analysis of dipeptidyl peptidase IV gene regulation in transgenic mice: DNA elements sufficient for promoter activity in the kidney, but not the intestine, reside on the proximal portion of the gene 5′-flanking region

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Received 10 July 2000; revised 24 August 2000; accepted 27 August 2000

Edited by Ned Mantei

Abstract The dipeptidyl peptidase IV (DPPIV) gene encodes a brush border membrane exopeptidase that is expressed in a tissue-restricted fashion. To examine the regulation of DPPIV transcription in various tissues in vivo, we examined the expression of DPPIV 5′-flanking region (promoter)–human growth hormone reporter constructs in transgenic mice. These mice exhibited cell-type specific reporter expression in kidney. Surprisingly, however, only very low to non-detectable levels of reporter were found in small intestine. These results indicate that DNA elements sufficient for DPPIV expression in kidney, but not intestine, reside in the 5′-flanking region of the gene.

Key words: Transgenic mouse; Brush border peptidase; Transcriptional regulation; Gene promoter; Promoter–reporter construct

1. Introduction

Dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5) is expressed in several tissues including small intestine, kidney, liver and lung. This hydrolase is a brush border membrane-associated amino terminus exopeptidase with high activity towards peptides with proline in the pentultimate position. In the intestine, this enzyme plays an important role in the digestion of proline-containing dietary proteins [1,2]. In kidney, studies using a rat strain lacking DPPIV indicate that the enzyme is required for urinary protein digestion prior to resorption by the proximal tubules [3]. In other tissues, the role of DPPIV is less clear although several functions have been proposed including lymphocyte activation, cell-basement membrane interaction, and modulation of hormonal activity [4–7].

Our laboratory has had a long-standing interest in brush border peptidases, their substrate specificities, and their regulation at the transcriptional level. We have demonstrated that a proline-rich diet induces DPPIV expression, mediated through increases in transcription [8]. Moreover, we have isolated the DPPIV gene 5′-flanking sequence, demonstrated that it has promoter activity in cultured cells, and characterized elements important for its activity in vitro [9,10]. A cognate hepatic nuclear factor-1 (HNF-1) binding site located between bases −147 and −135 appears to be especially important, as point mutations in this site reduce promoter activity to 5–10% of the non-mutated control [10]. We have also identified a consensus E-box motif in the DPPIV 5′-flanking sequence that binds upstream stimulatory factor, resulting in transcriptional activation [11]. The factors necessary for effecting tissue and cell-type specific expression of DPPIV transcription however, remain to be determined. Since DPPIV is expressed in several different tissues, its expression may be dependent upon different factors in different cell-types.

In order to examine tissue and cell-type specific DPPIV expression in vivo, we produced transgenic mice containing DPPIV promoter–reporter constructs. Analysis of these mice reveals appropriate transgene expression in the kidney, but a lack of expression in the intestine. These results suggest that the DPPIV 5′-flanking sequence contains elements sufficient to drive cell-type specific transcription in the kidney and further indicate that additional *cis*-elements are required to effect DPPIV expression in the intestine.

2. Materials and methods

RNA was extracted from mouse tissue using Tri reagent (Molecular Research Center, Cincinnati, OH, USA) as described [12]. Northern blots were prepared using 10 μg of total RNA per lane and hybridized to DPPIV [8], human growth hormone (hGH) [12], or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [12] probes. hGH immunohistochemistry was conducted using the silver-enhanced immunogold technique as previously [12]; tissues were fixed in Bouin's solution and sections were counter-stained with hematoxylin.

Because the human DPPIV gene was found to utilize multiple transcription start sites between bases −115 and −51 it is customary to number the promoter from the start of translation [9]. Human and mouse DPPIV promoter–hGH reporter transgenes were constructed using bases −952 to −41 of the human DPPIV promoter [9] and bases −1711 to −13 of the mouse DPPIV promoter [13] in conjunction with the hGH structural gene contained in the pOGH expression vector [12]. For construction of the human DPPIV–hGH transgene, the polymerase chain reaction (PCR) was used with primers M13A (5′-GCGCGTTGGCCGATTCATT) and DPP-7 (5′GAAGGATCC-CAGGCAGAAG, introduced *Bam*HI site underlined) to amplify the appropriate fragment from the M13mp19 *Xba*I–*Xba*I clone used for sequencing [9]. This fragment was digested with *Hin*dIII and *Bam*HI and cloned into the same sites of pOGH. The promoter–reporter transgene containing the human DPPIV promoter cloned into the 5′-untranslated region of the hGH structural gene was then retrieved with *Hin*dIII and *Eco*RI for transgenic mouse production. For preparation of the mouse DPPIV–hGH construct, C57Bl/6 mouse DNA

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Abbreviations: DPPIV, dipeptidyl peptidase IV; hGH, human growth hormone; HNF-1, hepatic nuclear factor-1; PCR, polymerase chain reaction

0014-5793/00/$20.00
PII: S0014-5793(00)02013-5

Human DPPIV Promoter Construct

gaaacttgccagcggcGAGTGACTCCACCGCCCGGAGCAGCGGTGCAGGACGCGCGTCTCCGCCGCCCGCGGTGACTTCTGCCTG**GGATCC**CAAGGCCC
AACTCCCCGAACCACTCAGGGTCCTGTGGACAGCTCACCTAGCTGCAATG GCT
Met Ala

Mouse DPPIV Promoter Construct

actcaggccgaaacttGGCGGCGAGCTCAGGGTGACTGCGTGCAGAGCAGCCGCGCAGGACGTCCGTCTCTGCGCGCAGTGACTTCTGCCTGCGCTCAA
GCTTCAGAGTTCAGTTTCAA**GGATCC**CAAGGCCCAACTCCCCGAACCACTCAGGGTCCTGTGGACAGCTCACCTAGCTGCAATG GCT
Met Ala

Fig. 1. Sequences of the human and mouse DPPIV promoter–hGH reporter constructs near the promoter–reporter transition sites. 5′-flanking region sequence is indicated using lowercase letters with the start of transcription of the longest known transcripts indicated by the bent arrows. DPPIV 5′-untranslated regions between the start of transcription and the *Bam*HI sites (bold) are indicated by uppercase letters. The *Bam*HI sites demarcate the transitions between the 5′-untranslated regions of the DPPIV promoters and the 5′-untranslated region of the hGH reporter genes. The first two codons are shown to mark the translation start sites of the reporters.

was amplified by PCR using primers MD-1 (5′-GAGATATCGTCGACTAACTACCCAAATGAGTTCTC) and MD-2 (5′-TTCATGGTCGGGCGGATCCTTGAAACTGAACTC), introduced *Sal*I and *Bam*HI sites underlined. Following digestion with *Sal*I and *Bam*HI, the fragment was cloned into the same sites of pOGH; the mouse DPPIV–hGH promoter construct was then retrieved with *Sal*I/*Eco*RI for transgenic mice production. The PCR fragments generated in these procedures were sequenced to assure faithful amplification. Fig. 1 illustrates the linkage between the promoter and reporter in both constructs.

All transgenic mice used in this study were produced at the University of California at Irvine (UCI) Transgenic Mouse Facility except for human strains 18 and 40, which were produced by the DNX corporation, Princeton, NJ, USA. Mice from the UCI facility were prepared using C57Bl/6×BALB/c F2 hybrid zygotes, mice from the DNX corporation were prepared using C57BL/6×SJL F2 hybrid zygotes. Founder mice were mated with C57Bl/6 mice to propagate lines and all mice used in these studies were from the founder to F2 generations. Transgene-bearing mice were detected using *Pst*I-digested tail DNA in blot analysis using the proximal 741 base *Pst*I fragment of the hGH structural gene as a probe.

3. Results

Past studies have confirmed the utility of hGH as a reporter gene in transgenic mice [12,14–16]. Its expression is detectable in whole tissues by Northern blot analysis and in histological sections by immunohistochemistry. Thus, this reporter was chosen for work with the DPPIV promoter–reporter constructs.

Five independent lines of human DPPIV–hGH promoter–reporter transgenics were obtained. These lines all contained 1–5 copies of transgene in their genome and the transgenics were similar to their normal litter mates in appearance and behavior. Mice from each line were dissected, RNA was extracted from tissues, and Northern analysis was performed to detect a hGH message (Fig. 2). In line 5, only very low or undetectable amounts of hGH reporter message was detected in any tissue, suggesting that the transgene became incorporated in a transcriptionally silenced region of the genome. Also, as expected, the non-transgenic control failed to express hGH message. The other four lines exhibited reporter expression in select tissues. High levels of hGH message were produced in the kidneys of lines 6, 18, and 40 and in the liver of strains 3, 6 and 18. Inappropriate expression of reporter was found in the colon of line 3 and the brain of line 6. Surprisingly however, low or no reporter expression was observed in the intestine of any line despite the fact that high levels of endogenous DPPIV are expressed in this tissue [17]. Because of this unexpected finding the blots were hybridized again to a GAPDH probe to ascertain that RNA was loaded in all lanes, typical results demonstrating this are included in Fig. 2.

In order to determine the tissue expression levels of endogenous DPPIV mRNA in the transgenics, Northern blots were prepared using RNA from two transgenic lines and hybridized to a DPPIV probe (Fig. 3). Here we found that the highest levels of DPPIV message were found in small intestinal RNA; kidney and liver in contrast, expressed lower but significant levels of message. Thus, these transgenic lines exhibited high levels of DPPIV message in the intestine, similar to non-transgenic animals [17].

Other than the HNF-1 site implicated mechanistically in DPPIV promoter activity [10], little conservation between

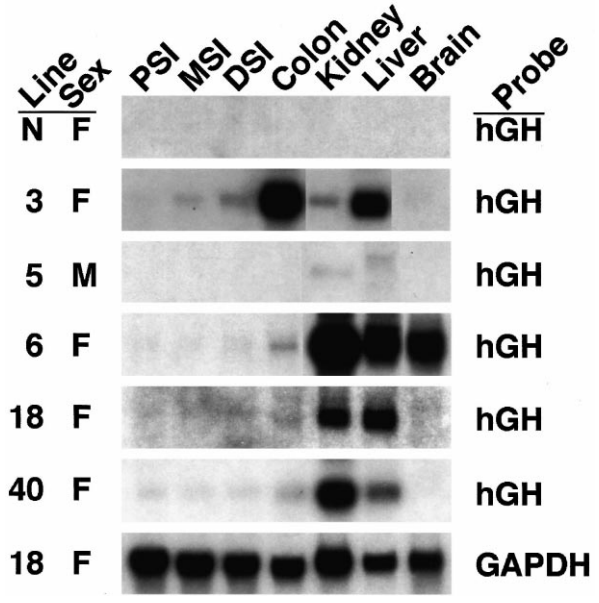

Fig. 2. Northern blot analysis of reporter expression in the human DPPIV promoter–hGH reporter transgenic mice. The line and sex of the animals are indicated on the left side of the figure (N indicates non-transgenic). RNA was extracted from the indicated tissues. PSI, MSI, and DSI indicate samples of proximal, middle and distal small intestine, respectively. Swatches of autoradiograms from the various tissues are shown, the first six autoradiograms were probed using the hGH reporter probe, the final autoradiogram is of a GAPDH control indicating RNA loading in all lanes.

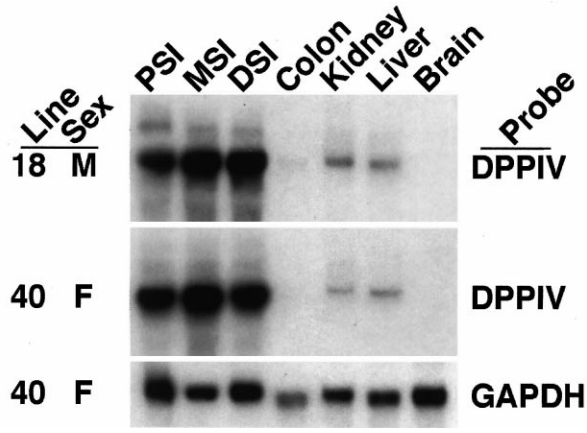

Fig. 3. Northern blot analysis of endogenous DPPIV message expression in human DPPIV promoter lines 18 and 40. Annotation is as in Fig. 2.

the 5′-flanking regions of mouse and human DPPIV is evident. This contrasts with other intestinal genes such as mucin MUC2 and sucrase–isomaltase in which at least the first 200 bases upstream of the start of transcription are well conserved [18,19]. Because of the differences present between the mouse and the human sequences, we considered the possibility that this lack of conservation was responsible for the absence of transgene expression in the intestine. To test this, we prepared a mouse DPPIV promoter–hGH reporter construct and prepared transgenics using this. We used longer segments of promoter (5′-flanking regions) and 5′-message untranslated regions with this construct as we believed that this had the best chance of containing elements necessary for intestinal expression. Four transgenic founders were obtained containing the mouse DPPIV promoter–hGH reporter construct. These mice, similar to the previous set, also all contained 1–5 copies of the transgene. Fig. 4 shows expression of reporter message in the tissues of this set of transgenics. These mice, similar to their counterparts with the human DPPIV promoter, expressed reporter message at high levels in their kidneys and livers. Also similar to the previous lines, these lines failed to express reporter in their intestines. Inappropriate reporter expression was not observed in colon or brain when the mouse 5′-flanking sequence was used. Moreover, careful comparison of Figs. 2 and 4 indicates no expression of reporter in the intestines when the mouse promoter was used while trace amounts appeared in some lines containing the human promoter–reporter construct.

DPPIV has been localized to the brush border membrane of the proximal tubules of the kidney nephron where it apparently functions in protein degradation prior to resorption [3,17]. Thus, in evaluating the specificity of reporter expression in the kidney, it is important to determine which cells express the reporter. We utilized mouse DPPIV–hGH line 2 for this purpose as it exhibits the highest level of reporter expression and is therefore most amenable to immunohistochemical analysis. We determined using this technique that reporter expression is localized to a select group of tubules in the kidney, in fact the proximal tubules (Fig. 5). These cells are distinguishable from the cells of the distal tubules which lack a brush border membrane and have smaller cell bodies. Cells of the distal tubules, in contrast, can be seen to lack reporter expression (Fig. 5). Also shown in Fig. 5 are results using a non-transgenic mouse kidney, in which immunostaining is absent. Thus, hGH reporter is expressed in a cell-type specific manner in the kidney of these mice.

4. Discussion

Our results indicate that the DNA elements required for expression of DPPIV in the kidney are located on the proximal portion of the 5′-flanking region of the DPPIV structural gene. Eight transcriptionally active mouse lines were produced using both human and mouse DPPIV promoter–hGH reporter transgenes and all of these lines expressed detectable reporter (hGH) message in their kidneys (Figs. 2 and 4). This expressed transgene product was found to be appropriately localized in cells of the proximal tubules of the nephron in mouse line 2, thus demonstrating that transgene expression in the kidney is cell type-specific as well. Surprisingly however, none of the lines expressed reporter in their intestine despite the fact that the highest level of endogenous DPPIV expression occurs in this tissue. This indicates that additional DNA binding elements are required for DPPIV expression in the intestine. The elements appear to located elsewhere than between bases −1711 and −13 of the mouse promoter, the longest construct used. Furthermore, it can be inferred from this data that a different set of DNA binding proteins (transcription factors) are used to achieve DPPIV expression in the kidney and intestine rather than altered levels of the same factors, as the factors required for transcription apparently bind to different elements in the two different tissues. Thus, while DPPIV is expressed in several cell types, its expression appears to utilize a relatively specific set of factors in the various cell types in which it is expressed.

Transgene expression in the liver generally paralleled expression in the kidney in the various lines, however there were some exceptions to this. Human line 40 and mouse line 2 expressed reporter at higher levels in the kidney while human line 3 expressed higher in the liver (Figs. 2 and 4). This phenomenon could be related to the site of integration of the transgene. For example, if integration occurred near an element that causes activation of gene expression in the liver then transgene expression in this tissue would be elevated. The same phenomenon is also likely to be responsible for trans-

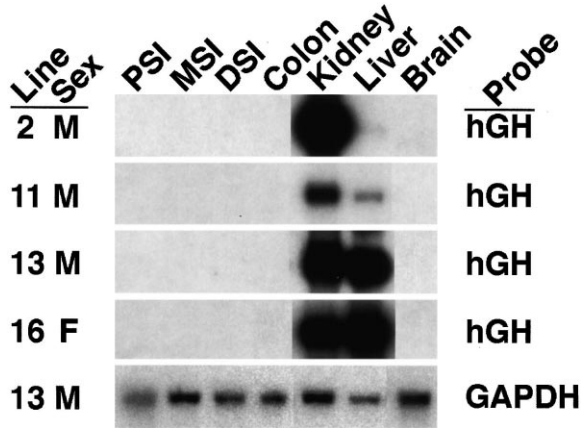

Fig. 4. Northern blot analysis of reporter expression in the mouse DPPIV promoter–hGH reporter transgenic mice. Annotation is as in Fig. 2.

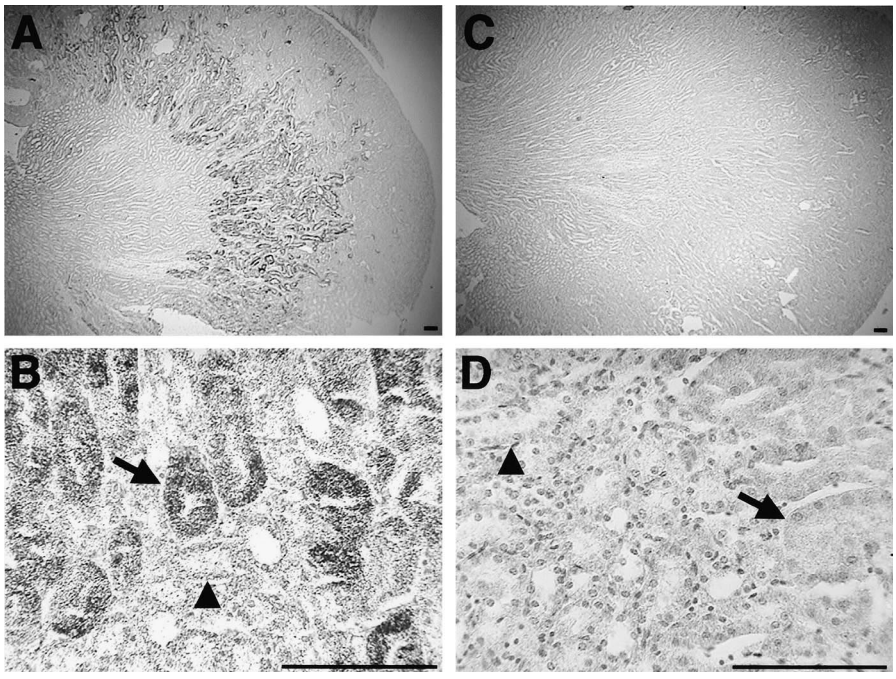

Fig. 5. Immunohistochemical analysis of hGH reporter expression in mouse DPPIV–hGH line 2 kidney. A and B are different magnifications of a transgenic kidney and C and D are from a non-transgenic litter mate. The arrows indicate proximal tubules (identified by their abundant cytoplasm and brush border membrane), the arrowheads indicate distal tubules. The bars are 100 μm.

gene expression in the colon in human line 3 and the brain in human line 6. It is also noteworthy that transcription seemed to be more tightly controlled when constructs containing the mouse promoter were used than when using constructs containing the human promoter; i.e. only transcription in the liver and kidney was observed. This is perhaps due to poor conservation between the human and mouse promoter sequences, causing incomplete suppression in inappropriate tissues with the human promoter. An alternative explanation is that longer regions of 5′-flanking sequence and 5′-untranslated message sequence were used to prepare the mouse promoter construct than were used in the human promoter construct.

The data presented in this paper indicate that DNA elements required for DPPIV gene transcription in the intestine are located elsewhere than in the proximal 5′-flanking region of the gene. This differs from results obtained to date with several other genes that are expressed in intestinal absorptive cells. Similar experiments using transgenic mice have demonstrated that elements required for tissue specific expression in the intestine are found on the 5′-flanking regions of the sucrase–isomaltase gene and fatty acid binding protein genes [14–16]. Our work with an intestinal goblet cell specific gene (*MUC2*) indicates that elements necessary for cell type specific expression in the small intestine, but not the colon, reside on the 5′-flanking region [12]. Thus, the regulation of DPPIV is dissimilar in this regard from other intestine-specific genes that have been studied in this detail in vivo. It is worth noting that the second intron of the DPPIV gene is approximately 20 kb and genes are known where regulatory sequences are located in introns or even in the 3′-flanking region [20–22]. Moreover, sequences located many kb from the start of transcription have been shown to be important in tissue-specific gene regulation [23,24]. The elements that control DPPIV gene expression in the intestine could be located in any of these locations.

Comparison of the mouse and human DPPIV 5′-flanking sequences indicates an absolute conservation of the HNF-1 element [10]. This occurs despite a lack of conservation elsewhere in the proximal 5′-flanking sequence. HNF-1 transcription factors (HNF-1α and HNF-1β) are expressed in multiple tissues including intestine, liver, and kidney [25,26] and are therefore present in most if not all of the tissues that express DPPIV [17]. Previous work in our laboratory has demonstrated that mutations in this site diminish DPPIV promoter activity to 5–10% of unmutated controls [10]. Moreover, increases in HNF-1α are correlated with increases in DPPIV levels during differentiation in Caco-2 cells [27]. Thus, the HNF-1 site appears to be very important for DPPIV promoter function even though it may not be the sole determinant of cell-type specificity of the promoter.

In summary, we have demonstrated using promoter–reporter constructs in transgenic mice that elements required for cell type-specific expression of DPPIV in the kidney are located on the 5′-flanking region and that a different set of elements are utilized in the intestine. These results indicate therefore, that the DPPIV promoter utilizes multiple sets of DNA binding elements and transcription factors for expression in various tissues. Additional studies will be required to determine the unique combinations of transcription factors and DNA binding elements required for DPPIV gene expression in each of the individual tissues.

Acknowledgements: This work was supported by USPHS Grant No. DK17938 and by the Department of Veterans' Affairs Medical Research Service. We also thank Mr. Tom Fielder of the University of California at Irvine Transgenic Mouse Facility and Dr. Rick Huntress of the DNX Corporation for their assistance in preparing the transgenic mice.

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