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A transgenic mouse line with α-1,3/4-fucosyltransferase cDNA: production and characteristics

HIROYA OBAMA[1], TADASHI KANAME[1,2], AKIHARU SUDOU[1,3], TOYOKO YANAGIDA[1,4], SHINYA IKEMATSU[1], MASAYUKI OZAWA[1], HIROKI YOSHIDA[5], REIJI KANNAGI[6], KEN-ICHI YAMAMURA[2] and TAKASHI MURAMATSU[1,3]*

Departments of [1]Biochemistry, [4]Ophtalmology and [5]Pathology, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890, Japan
[2]Department of Developmental Genetics, Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, 4-24-1 Kuhonji, Kumamoto 862, Japan
[3]Department of Biochemistry, Nagoya University School of Medicine, 65 Tsurumaicho, Showaku, Nagoya 466, Japan
[6]Department of Pathology, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusaku, Nagoya 464, Japan

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cDNA of human α-1,3/4-fucosyltransferase (Fuc-TIII) was placed under the control of the chicken β-actin promoter and cytomegalovirus enhancer, then introduced into male pronuclei of fertilized mouse eggs. A transgenic mouse line thus obtained exhibited enhanced expression of Le^x (4C9) antigen in endothelial cells located in the glomerulus, sinusoidal capillaries of the liver and capillaries of the heart. Furthermore, in the transgenic mice, sialyl dimeric Le^x (FH6) and sialyl Le^a (2D3) antigens were strongly expressed in the glomerular endothelial cells.

Keywords: transgenic mice, α-1,3/4-fucosyltransferase, Le^x antigen

Introduction

Transgenic mice with overexpressed glycosyltransferase genes and mice homozygous for disrupted glycosyltransferase genes are highly useful in analysing the *in vivo* function of specific carbohydrate linkages [1–5]. These mice can also be utilized as sources to isolate glycoproteins with altered carbohydrate structures.

We previously produced transgenic mice with an ectopically expressed α-1,3-galactosyltransferase gene [2]. Even though the introduced enzyme gene was expressed in embryos, it did not severely hinder embryogenesis. The transgenic adult mice had the tendency to show certain abnormalities such as increased protein content in the urine.

This communication deals with the production of a transgenic mouse line with human α-1,3/4 fucosyltransferase (Fuc-TIII) cDNA, which can form Le^x, Le^a, sialyl Le^x and sialyl Le^a linkages [6]. Le^x structure is considered to be involved in compaction during early mouse embryogenesis [7, 8] and also in enhancement of cell-substratum adhesion [9, 10]. Sialyl Le^x and sialyl Le^a serve as ligands for selectins, carbohydrate recognizing cell adhesion molecules involved in leukocyte trafficking [11–14]. Thus, transgenic mice with enhanced expression of these antigens are expected to be valuable in diverse experiments concerning physiological and pathological roles of these sugar linkages.

Materials and methods

Construction of the transgene

Human α-1,3/4-fucosyltransferase (Fuc-TIII) cDNA [6] was kindly given by Dr J.B. Lowe. The cDNA in pCDM8 was digested with *Xho* I/*Nae* I and separated by TAE agarose gel electrophoresis. The isolated fragment was digested with *Nde* I, and the *Nde* I/*Nae* I fragment was isolated by TAE agarose gel electrophoresis and a Geneclean II kit (Bio. Lab.). The fragment was blunt ended with T4 DNA polymerase and was inserted into

*To whom correspondence should be addressed.

the pCAGGS expression vector [15] with the chicken β-actin promoter and cytomegalovirus enhancer at the *Eco* RI site after fill-in reaction with T4 DNA polymerase. The completed plasmid (Fig. 1) was purified by means of $CsCl_2$ gradient centrifugation, and expression of the enzyme activity was confirmed by transfection into COS cells using DEAE-dextran [16]. The DNA fragment containing the transferase cDNA as well as the enhancer, the promoter and the β-globin 3′-flanking sequence was excised from the vector sequence by digestion with *Hind* III/*Sal* I, separated by electrophoresis in TAE agarose gel and purified using a Geneclean II kit.

Transgenic mice

Transgenic mice were produced essentially as described by Yamamura *et al.*, [17]. DNA was microinjected into male pronuclei of $(C_{57}BL/6J \times DBA/2)F_1$ (BDF_1) mice. The microinjected fertilized eggs were then transferred to pseudopregnant recipient ICR mice. Transgenic mice were identified by polymerase chain reaction (PCR) [18] and Southern blots of the DNA extracted from mouse tail biopsies as described previously [17]. Transgene-carrying mice (F_0) were then mated with BDF_1 mice. Pups born from these crosses were screened for the transgene; F_1 mice which carried it were then mated with $C_{57}BL/6J$. F_2 mice thus produced were used for analysis.

Immunohistochemical detection of carbohydrate antigens

Specimens were fixed with buffered formalin and embedded in paraffin. The 5 μm sections were deparaffinized with xylene and endogeneous peroxidase was blocked by incubation with 0.3% hydrogen peroxide in absolute methanol at room temperature for 30 min. After rehydration in graded ethanol, sections were washed with Dulbecco's phosphate-buffered saline (PBS), and then with 1% bovine serum albumin (BSA) in PBS. The sections were incubated with monoclonal antibodies diluted 100 ~ 200-folds with PBS containing 1% BSA at 4 °C overnight. The antibodies used were anti-4C9 (rat IgM) [10, 19], anti-FH6 (mouse IgM) [20] and anti-2D3 (mouse IgM) [21]. After washing with PBS, sections were then incubated with goat anti-rat IgG (Vector Laboratories) or goat anti-mouse IgM (Vector Laboratories). The bound IgG was visualized by streptoavidin-biotin complex kit (Vector Laboratories) and 3,3′-diaminobenzidine tetrachloride. The stained sections were counterstained with Haematoxylin and mounted. Control stainings in which the first antibody was omitted, were negative in all cases.

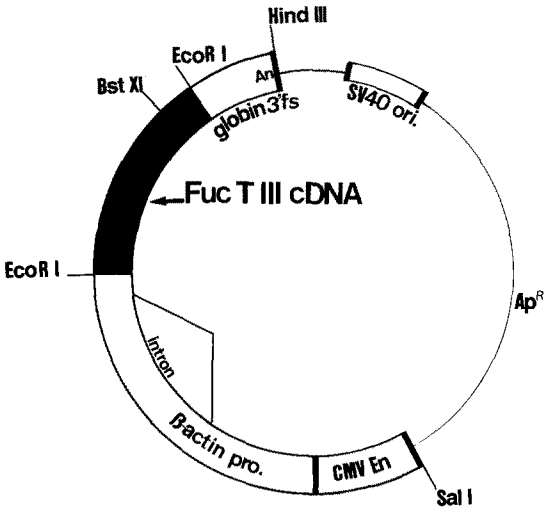

Figure 1. Construction of the pCAGGS expression vector containing α-1,3/4 fucosyltransferase cDNA. Abbreviations: β-actin pro., chicken β-actin promoter; CMV En, cytomegalovirus enhancer; Fuc-TIII cDNA, α-1,3/4-fucosyltransferase cDNA; globin 3'fs, rabbit β-globin 3′-flanking sequence; An, a polyadenylation signal; SV40, simian virus 40; ori, origin of DNA replication; Ap^R, ampicillin resistant gene.

Results

Production of mice with the α-1,3/4-fucosyltransferase transgene

The cDNA of human α-1,3/4-fucosyltransferase (Fuc-TIII) was placed under the control of chicken β-actin promoter and cytomegalovirus enhancer, and was injected into 157 fertilized mouse eggs, resulting in 19 pups of which only one carried the transgene as revealed by PCR and Southern blotting. Using the transgenic mouse as the founder, we established the transgenic mouse line, resulting in production of F_2 mice (Fig. 2). Polymerase chain reaction after reverse transcription, followed by Southern blotting, verified that the lung, liver and kidney indeed expressed human Fuc-TIII mRNA, which was not detected in wild-type mice (Fig. 3). So far transgenic mice have revealed no distinct abnormalities.

Increased expression of Le^x, sialyl dimeric Le^x and sialyl Le^a antigens in transgenic mice

We analysed the expression of Le^x antigen in tissue sections of the transgenic mice and in those of wild-type mice. The organs so far examined were the kidney, heart, liver, lung and spleen. Monoclonal antibody 4C9 was used to detect Le^x antigen [19]. As summarized in Table 1, increased Le^x expression was clearly observed in the glomerular endothelial cells of the kidney (Fig. 4 A and D), endothelial cells of sinusoidal capillaries of the liver (Fig. 4, B and E) and those of capillaries of the heart (Fig. 4, C and F). In the kidney, renal tubules expressed Le^x intensely both in the transgenic and wild-type mice. However, the glomerular endothelial cells were stained by 4C9 only very faintly in the wild-type mice, while in the transgenic mice, they were distinctly stained (Fig. 4 A and D). Endothelial cells in other renal regions were not strongly stained. Thus,

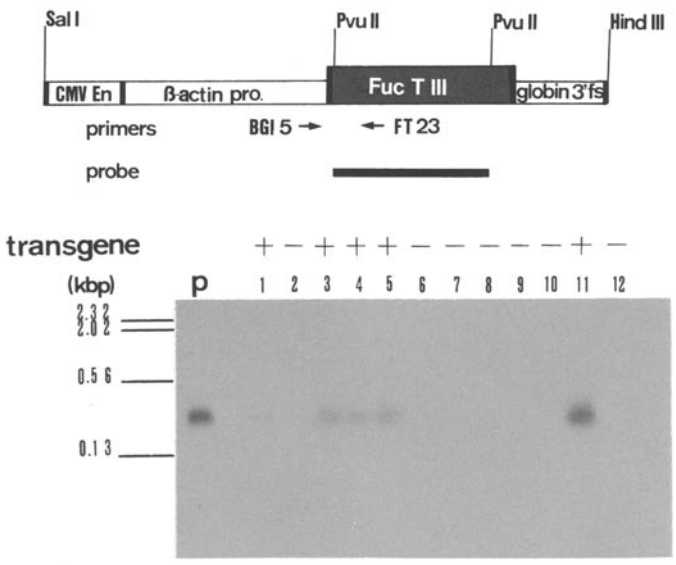

Figure 2. Southern blot analysis of transgene amplified by PCR. BG1 (5′-GCTAACCATGTTCATGCCTTC-3′) and FT23 (5′-CATGTCCATAGCAGGATCAG-3′) were used as primers, and DNA isolated from tail biopsy was used as templates. A fragment (926 bp) from Fuc-TIII cDNA (PVUII/PVUII) labelled with [32P] was used as a probe. The PCR conditions were 93 °C 1 min, 58 °C 2 min and 72 °C 3 min, and reaction was repeated 30 times. The PCR samples were obtained from crosses of F1 (BDF_1 with heterozygous transgene) with $C_{57}BL/6J$ and segregation of transgene positive and negative mice was expected to be 1:1. The hybridizing PCR product (numbers 1, 3, 4, 5, 11) had the expected size of 0.25 kb. P; positive control. An expression vector pCAGGS with inserted Fuc-TIII cDNA (Fig. 1) was used as a PCR template. Abbreviations: CMV En, cytomegalovirus enhancer; β-actin pro., chicken β-actin promoter; Fuc-TIII cDNA, α-1,3/4-fucosyltransferase cDNA; globin 3'fs, rabbit β-globin 3′-flanking sequence.

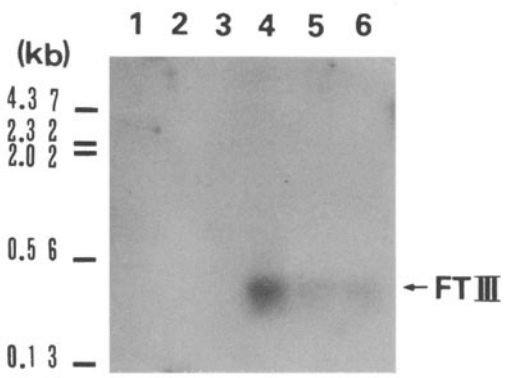

Figure 3. PCR after reverse transcription to detect human Fuc-TIII mRNA in transgenic mice. Total RNA (0.2 μg) extracted from organs of wild-type or transgenic mice by the acid guanidium-thiocyanate method [25] was reverse transcribed using RT primer pd $(N)_6$ (Pharmacia LKB Biotechnology) and M-MLV Reverse Transcriptase (GIBCO BRL) as described elsewhere [26]. PCR was performed using sense primer (5′-AGCCAAGCCACAATGGCCAT-3′) and antisense primer (5′-TTGAAGTATCTGTCCAGGGC-3′). The amplification procedure consists of 35 cycles (93 °C, 1 min; 58 °C, 1 min; 72 °C, 1 min). The PCR product was analysed by Southern blotting using Fuc-TIII probe as described in the legend of Fig. 2. Lanes 1, 2, 3, wild-type mice ($BDF_1XC_{57}BL/6$); lanes 4, 5, 6, transgenic F_2 mice; lanes 1, 4, the lung; lanes, 2, 5, the liver; lanes 3, 6, the kidney. FTIII indicates the position where Fuc-TIII PCR product migrates.

increased Le^x antigen expression in the transgenic mice has so far been observed in certain endothelial cells.

We also examined the expression of FH6 antigen (sialyl dimeric Le^x antigen) and 2D3 antigen (sialyl Le^a antigen) in transgenic and wild-type mice (Table 1). Both antigens were strongly expressed in the glomerular endothelial cells of transgenic mice, but only faintly in those of wild type mice (Fig. 5). These antigens were not expressed in renal tubules. Thus, increased antigenic expression in transgenic mice was more clearly observed for these antigens than for Le^x antigen. In other tissues so far examined, we could not detect the difference in expression of sialyl dimeric Le^x antigen and sialyl Le^a antigen between wild-type and transgenic mice (Table 1).

As to the expression of Le^x, sialyl Le^x and sialyl Le^a in splenic lymphocytes and neutrophils, no significant difference was observed between the transgenic mice and wild-type mice.

Discussion

We have described, for the first time, the production of transgenic mice expressing an exogeneously introduced fucosyltransferase gene, more precisely α-1,3/4-fucosyltransferase (*Fuc-TIII*) gene. Even though the importance of production of transgenic mice with a glycosyltransferase gene is well known, transgenic mice with ectopically expressed α-1,3/4-galactosyltransferase produced by us [2] is the first example of such mice. The second example is cell-surface β-1,4-galactosyltransferase [3]. To the best of our knowledge, the mice reported here is the third example. We also tried to produce transgenic mice with α-1,3-fucosyltransferase (*Fuc-TIV*) gene using the same procedure. However, all efforts to produce such mice have been unsuccessful. Previously, we observed that transfection of L cells with Fuc-TIV or Fuc-TIII resulted in increased cell-substratum adhesion [10]. We reasoned that increased fucosylation of integrin-associated molecules enhanced integrin action. Alternatively, decreased sialylation as the result of increased fucosylation [22] may increase cell-substratum adhesion. The increased cell-substratum adhesion was more strongly observed in Fuc-TIV-transfected cells than in Fuc-TIII-transfected cells. Thus, it is possible that transgenic mice with Fuc-TIV are difficult to obtain, since

Table 1. Expression of Le^x and related antigens in transgenic and wild-type mice

	Staining reaction with					
	4C9 (anti-Le^x)		*FH6 (anti-sialyl Le^x)*		*2D3 (anti-sialyl Le^a)*	
	Transgenic mice	*Wild-type mice*	*Transgenic mice*	*Wild-type mice*	*Transgenic mice*	*Wild-type mice*
Kidney						
Glomerular endothelial cell	++	+	++	+	++	+
Proximal tubule	++	++	−	−	−	−
Distal tubule	+[h]	−	−	−	−	−
Collecting tubule	++	++	−	−	−	−
Endothelial cell other than the glomerulus	±	±	++	++	++	++
Liver						
Sinusoidal endothelial cell	++	+	++	++	++	++
Central vein	+	+	+	+	+	+
Portal vein	+	+	+	+	+	+
Heart						
Myocyte	−	−	−	−	−	−
Capillary endothelial cell	++	+	++	++	++	++
Lung	+[al]	+[al]	++[end]	++[end]	++[end]	++[end]

−, negative; +, weak; ++, moderate ~ strong; h, some distal tubule; al, alveolar cell; end, endothelial cell.
Staining was performed in two transgenic mice ($BDF_1XC_{57}BL/6J$, 16-week-old, male and female) and three wild type mice (two $BDF_1XC_{57}BL/6J$ mice, 16–20-week-old male and female, and one BDF1, 16-week-old male). The staining pattern described in the Table was reproducibly observed in different mice.

alteration of cell behaviour is more prominent in Fuc-TIV-expressing cells than Fuc-TIII-expressing cells. Generally speaking, production of transgenic mice with expressed glycosyltransferase genes which specify sugar linkages involved in intercellular recognition may be a difficult task. When the expression is intense, such embryos may die *in utero*. Viable mice may only be obtained when moderate or restricted expression occurs. In other words, the difficulty in obtaining Fuc-TIV and Fuc-TIII transgenic mice indicated that regulated fucosylation is important for normal embryogenesis.

Even though the β-actin promoter was used, expression of fucosylated antigens was restricted to the tissues in which the antigens are naturally expressed. This predicted the idea that the expression of fucosylated antigens examined in this study might be determined by the presence of precursor structures. The lack of correlation between Fuc-TIII mRNA expression and antigen expression between different organs (Fig. 3 and Table 1) supports the proposal.

In the glomerulus, not only Le^x antigen but also sialyl dimeric Le^x and sialyl Le^a antigen exhibited increased expression. However, in sinusoidal endothelial cells of the liver and heart endothelial cells, Le^x antigen increased in transgenic mice, while sialyl dimeric Le^x and sialyl Le^a antigen did not. Again, acceptor structure to form sialyl dimeric Le^x and sialyl Le^a may be present only in limited amounts of these sites.

Although no abnormality has been observed in the transgenic mice so far, the possible effects of the increased antigenic expression on inflammation and infection should be examined, especially because increased antigenic expression is observed in certain endothelial cells. Since in inflammatory states the initial stage of leukocyte-endothelial cell interactions is mediated by selectins, carbohydrate-recognizing cell adhesion molecules [11–14] and carbohydrate alteration in endothelial cells can change inflammatory reactions.

As a newly identified role of sialyl Le^x and sialyl Le^a, they were reported to participate in angiogenesis, especially in tube formation of bovine capillary endothelial (BCE) cells. Namely, in a BCE cell, sialyl Le^x and/or sialyl Le^a binds to E-selectin in capillary morphogenesis [23]. Angiogenesis occurs during development, and in adult life during wound healing and endometrial proliferation. Abnormal capillary growth underlies many disease states in which endothelial cells are stimulated to migrate into the perivascular space and form new capillaries [24]. The transgenic mouse we present here is a good model for the investigation of the role of glycoconjugates in angiogenesis occurring in tumour development, some inflammatory states and wound healing.

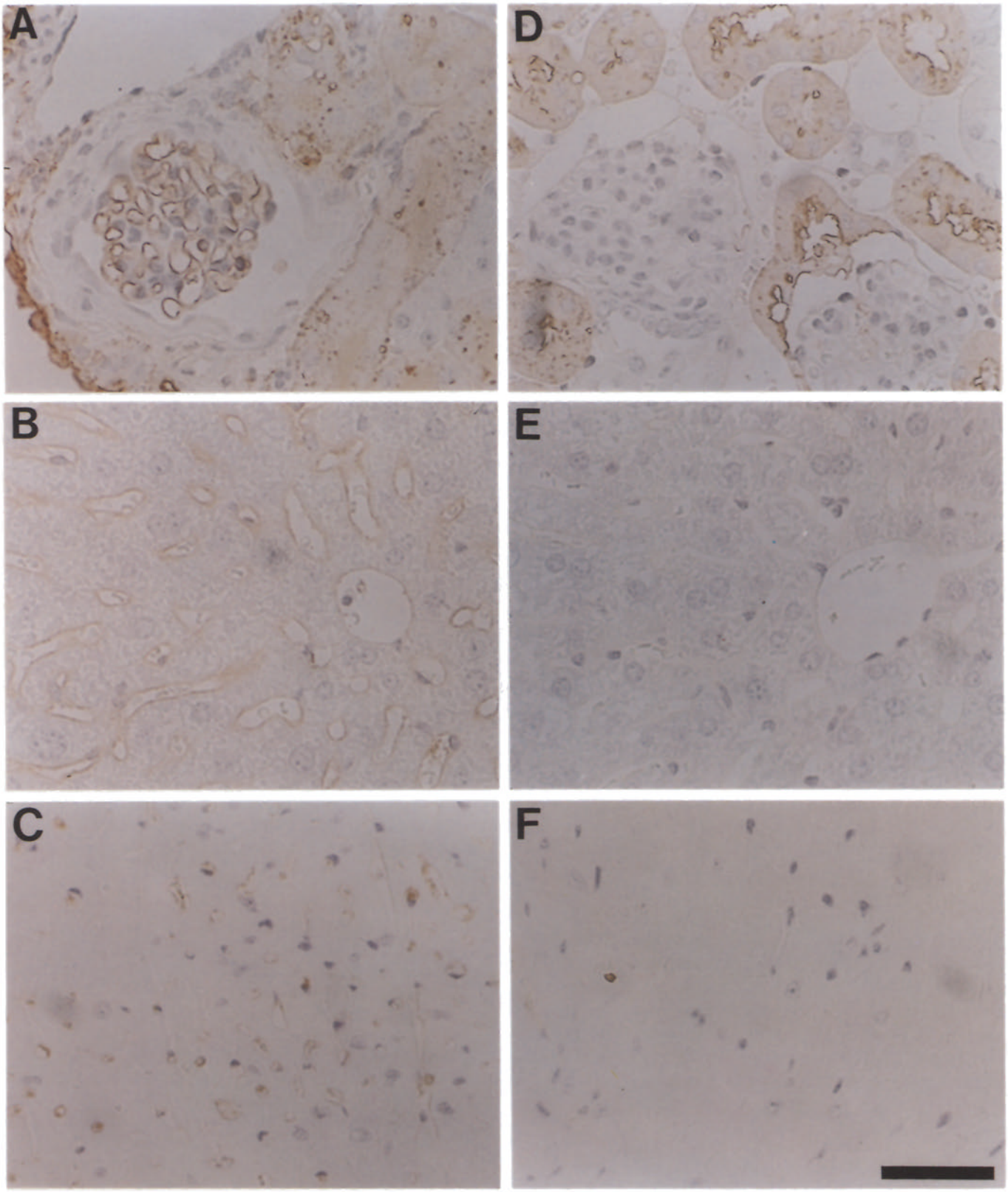

Figure 4. Le^x expression in *Fuc-TIII* transgenic mice and wild-type mice. Tissue section was stained by 4C9. Tissue was taken from a 16-week-old female transgenic mouse (A–C) ($BDF_1XC_{57}BL/6J$) and a 16-week-old female wild-type mouse (D–F) ($BDF_1XC_{57}BL/6J$). Bar 50 μm. A and D, kidney; B and E, liver; C and F, heart.

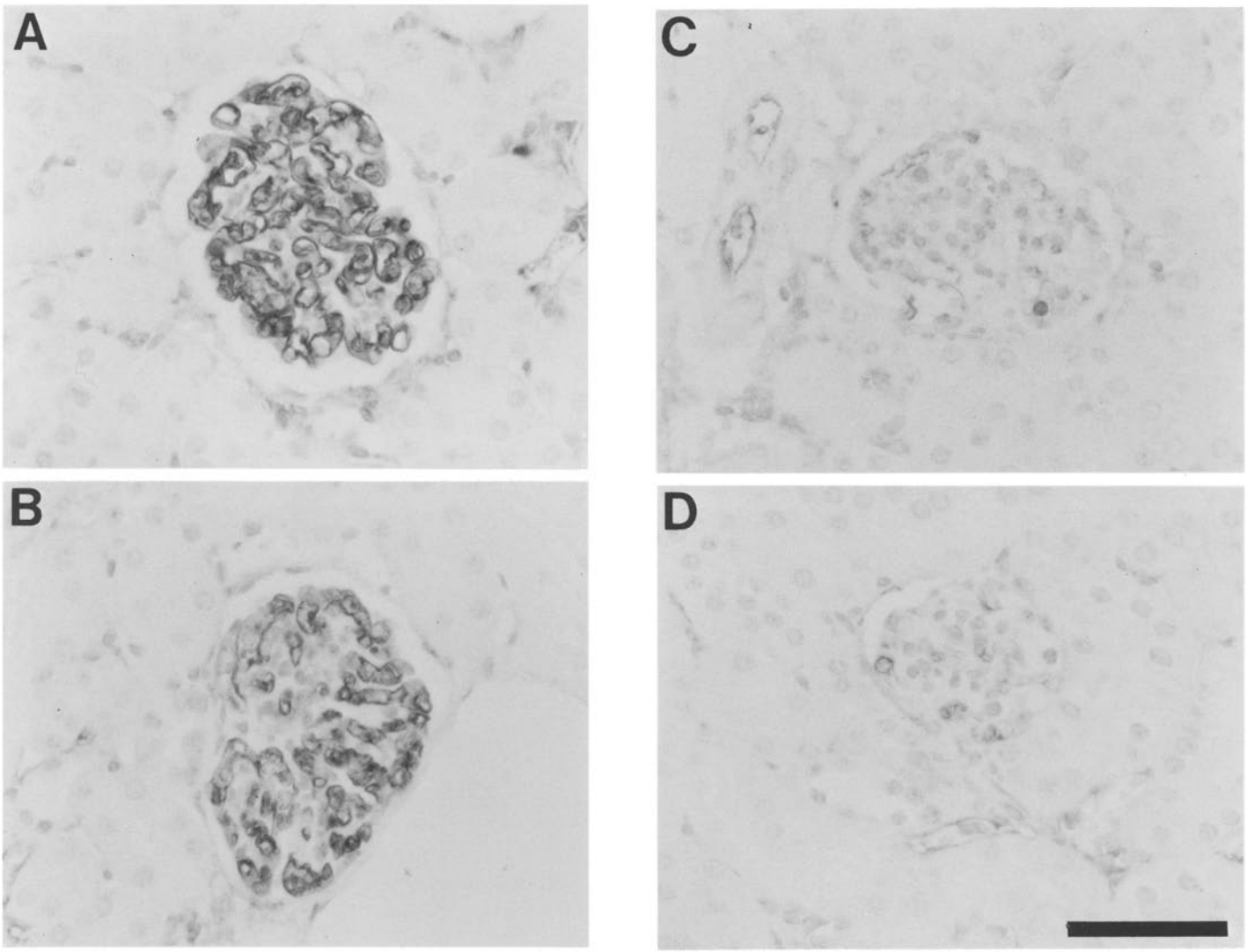

Figure 5. Sialy Le^x and sialyl Le^a expression in *Fuc-TIII* transgenic mice and wild-type mice. Tissue section was stained by FH6 or 2D3. Tissue was taken from a 16-week-old female transgenic mouse (A and B) ($BDF_1XC_{57}BL/6J$) and a 16-week-old female wild-type mouse (C and D) ($BDF_1XC_{57}BL/6J$). Bar 50 μm. A and C, FH6 antigen; B and D, 2D3 antigen.

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