Human renin in transgenic mouse kidney is localized to juxtaglomerular cells

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To examine whether expression of human renin in the transgenic mouse kidney is regulated in a cell-specific manner, we have characterized monoclonal antibodies against human renin and determined the renin-production site by immunohistochemistry. By using a monoclonal antibody specific for human renin, A6-11-6, we demonstrated that human renin in the transgenic mouse kidney is localized to the juxtaglomerular cells of afferent arterioles.

INTRODUCTION

Renin is an aspartic proteinase which hydrolyses angiotensinogen to form the decapeptide angiotensin I. Angiotensin-converting enzyme removes a dipeptide from the C-terminal end of angiotensin I to yield a vasoactive octapeptide, angiotensin II. The reaction catalysed by renin is the rate-limiting step in the renin–angiotensin system, and thus the enzyme plays an important role in the regulation of blood pressure and possibly in the pathogenesis of hypertension.

It is well known that the major site of human renin synthesis is the kidney, where synthesis has been localized to the juxtaglomerular (JG) cells [1–3]. Although considerable attention has been given to the study of cell-specific regulation of renin-gene expression, such investigations have been limited by the failure in establishing JG-cell cultures, which is due primarily to the extremely low proportion of these cells in the kidney (content 0.1% or less of total renal cells).

Transgenic animals provide an excellent model system for analysing the control of gene expression [4]. We have recently generated transgenic mice harbouring the human renin gene, which is expressed predominantly in its kidney [5]. An important question regarding the human renin gene is whether expression of such a transgene in the kidney is regulated in a cell-specific manner. In the present study, we have localized human renin production to the kidney cells of the transgenic mouse.

EXPERIMENTAL

Transgenic mice

Transgenic mice were derived by the microinjection of fertilized mouse eggs with the cloned human renin gene as described previously [5].

Renin and its inhibition assays

Four monoclonal antibodies, A6-11-6, A6-11-6-2, A6-69-30 and 12-12, obtained by immunizing mice with purified recombinant human active renin, were generously provided by Dai-ichi Radioisotope Co. Ltd. (Tokyo, Japan). The ability of these antibodies to inhibit the enzyme activity of human renin were tested. Recombinant human active renin (1 µg/ml) was used for

the inhibition assay and was incubated with various amounts of each monoclonal antibody at 4 °C for 10 h before the addition of partially purified pig antihypertensinogen as substrate, which had no detectable renin activity. After 20 min of further incubation at 37 °C, renin activity was measured by radioimmunoassay (r.i.a.) and expressed as the amount of angiotensin I generated/h and compared with the level of angiotensin I generated in the absence of antibodies.

Each kidney from transgenic and non-transgenic mice was homogenized in 4 vol. of phosphate-buffered saline (PBS) containing the proteinase inhibitors 5 mM-EDTA, 5 mM-sodium tetrathionate (STT) and 5 mM-phenylmethanesulphonyl fluoride (PMSF), and the supernatant were assayed for renin activity after centrifugation at 100 000 g at 4 °C. Inhibitory activity, with increasing dilution of A6-11-6 antibody, was measured by r.i.a. as described above.

Immunohistochemical procedure

Mice were perfused through the heart with 4% (w/v) paraformaldehyde in 0.1 M-phosphate buffer, pH 7.3. The kidney was removed and immersed in the same fixative for 4 h at 4 °C. After rinsing in PBS, the tissue was cryoprotected with 10, 15 and 20% (w/v) sucrose in PBS for 2 h in each step, frozen, and sectioned at 8 µm thickness. The sections were air-dried and then incubated with PBS containing 1% BSA for 1 h. The monoclonal antibody A6-11-6, specific for human renin, was diluted to 1:800 in PBS and applied to the sections in a humid atmosphere at room temperature for 2 h. The specimens were washed with PBS and incubated with the secondary antibody, goat anti-mouse IgG (dilution 1:100 in PBS), for 2 h. After washing with PBS, they were treated with the serum of peroxidase–anti-peroxidase–mouse IgG complex diluted 1:100 in PBS for 2 h. The peroxidase reaction was revealed with 0.02% diaminobenzidine and 0.001% H₂O₂ for 5–10 min [6]. Control sections were handled at the same time in an identical manner and incubated with PBS instead of PBS-diluted A6-11-6 antibody.

RESULTS AND DISCUSSION

The ability of four monoclonal antibodies, A6-11-6, A6-11-6-2, A6-69-30, and 12-12, to inhibit the enzyme activity of purified recombinant human active renin was studied (Fig. 1). A6-11-6

Abbreviations used: JG, juxtaglomerular; PBS, phosphate-buffered saline; PMSF, phenylmethanesulphonyl fluoride; r.i.a., radioimmunoassay; STT, sodium tetrathionate; SV40, simian-virus 40.

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Fig. 1. Inhibition of human renin activity by monoclonal antibodies

Renin activity was measured by r.i.a. as the rate of angiotensin I production, and results were expressed as the percentage of angiotensin I generated/h as compared with the level of angiotensin I generated in the absence of antibodies. Monoclonal antibodies are indicated as follows: ■, A6-11-6; □, A6-11-6-2; ▽, A6-69-30; ▼, 12-12.

Fig. 2. Human active renin in transgenic-mouse kidneys

Each kidney extract from transgenic (hRN8-12) (○, △) and non-transgenic (●) mice was subjected to renin assay with increasing dilutions of A6-11-6 antibody. Recombinant human active renin (■) was used as a control for the enzyme reaction. Angiotensin I generated was measured by r.i.a., and results were expressed as described in the legend to Fig. 1.

antibody completely inhibited the human renin activity, whereas the others partially inhibited it, and the antibody did not affect the enzyme activity of mouse submandibular renin, even at the highest concentration (results not shown). These results indicated that A6-11-6 antibody was highly specific for human renin and was therefore chosen as the antibody for the analyses shown below.

Our previous work suggested, by direct r.i.a. using a monoclonal antibody recognizing the active site of human renin, that human renin is processed to the mature form in hRN8-12 mouse kidney [5]. To monitor whether the transgenic human renin has an ability to hydrolyse angiotensinogen, the angiotensin I-generating activity in kidney extracts from hRN8-12 and non-transgenic mice were examined using A6-11-6 antibody by r.i.a. as described in Fig. 1. Fig. 2 shows that renin activities of the

Fig. 3. Immunohistochemical analysis of human renin in transgenic- and non-transgenic-mouse kidneys

Frozen sections were treated with PBS (panels A and C) and with A6-11-6 antibody diluted by PBS (panels B and D). JG cells are indicated by an arrowhead. Panels A and B, non-transgenic-mouse kidneys; panels C and D, transgenic-mouse kidney (hRN8-12). Magnification $\times 250$.

kidney extracts from two hRN8-12 mice were inhibited by 10% in the presence of the antibody, whereas there was no effect of the antibody on the enzyme activity of the extract from non-transgenic mouse. Although our previous result showed that human renin in hRN8-12 kidney was accumulated to a 7.5-fold higher extent than in human kidney [5], the present results suggested that the transgenic-mouse kidney contains one-tenth the human renin contained in the non-transgenic-mouse kidney and that the mature form of human renal renin in the mouse functions as an active enzyme.

In order to identify the kidney-cell type that synthesizes human renin, we used the hRN8-12 mouse line for immunohistochemical analysis, as the transgene in this line was shown to be expressed predominantly in the kidney, where its gene product can act as a functional enzyme (Fig. 2). Using A6-11-6 antibody, we found intense immunostaining only in the epitheloid JG cells, as shown in Fig. 3, panel D. No human renin was detectable in other tubular structures or in the medullary region of the kidney. Control sections (Fig. 3, panels A, B and C) exhibited no positive staining in JG cells, whereas the antisera against mouse renin from submandibular gland stained these cells in both transgenic and non-transgenic mice (results not shown). Connective tissues and basement membranes were faintly stained in both transgenic- and non-transgenic-mouse kidneys, possibly because goat anti-mouse IgG was used as the secondary antiserum for the immunoreactions.

The mammalian JG apparatus is located on the hilar parts of the afferent arterioles [7]. Much evidence from immunohistochemical studies has shown that synthesis of human and mouse renins in the kidney is confined to JG cells [1,2,8]. Further analysis by hybridization *in situ* has confirmed this cell-specificity of renin-gene expression in human kidney [3]. Although the interlobular arteries of human and mouse kidneys have also been shown to synthesize renin [1,8], the human gene product was undetectable in these regions of our transgenic-mouse kidney.

Hybridization *in situ* to cellular RNA is an excellent method of localizing a small subset of cells that express a gene of interest in transgenic mice. By contrast, an immunohistochemical study using antibodies is advantageous at detecting and localizing a transgene product as a functional protein. Indeed, it has recently

been demonstrated that the simian-virus-40 (SV40) T-antigen gene fused to mouse *Ren-2* promoter is expressed in JG cells of the transgenic mouse kidney using either an antisense RNA probe or antibody specific for SV40 T antigen [9]. All strains of inbred mice have a common renin gene, *Ren-1* and some strains have an additional one, *Ren-2*. Although the *Ren-1* and *Ren-2* transcripts have greater than 96% sequence identity [10], they could be quantitatively distinguished by primer extension analysis [11]. In the case of transgenic mice with the *Ren-1* genetic background carrying the cloned *Ren-2*, the transgene expression was examined by this method and the organ-specific accumulation of its mRNA was observed [12–14]. However, it has not been investigated whether synthesis of the transgene product in the kidney was regulated in a cell-specific manner. In the present study, using the monoclonal antibody specific for human renin, we have demonstrated that human renin in the transgenic mouse kidney can function as an active enzyme and that it is produced predominantly in JG cells, although the kidney is composed of multiple cell types. From these results, we conclude that cell-type-specific expression of the human renin gene can be preserved, even when the human gene is introduced into a foreign chromosome of the mouse.

We are grateful to Kuniko Katsuki, Kazuhiro Kaseda, Kazuki Nakao, and Yoshitaka Hiroshima taking care of the animals. We thank Dr. Robert Di Nicolantonio for critical reading of the manuscript before its submission. This work was supported by grants from the Ministry of Education, Science and Culture (Japan) and Chichibu Cement Co. Ltd., Tokyo, Japan.

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Received 8 April 1991/29 May 1991; accepted 12 June 1991